THE INTERNAL SECRETIONS IN GROWTH AND DEVELOPMENT OF AMPHIBIANS

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While up to 1910 the higher vertebrates were used predominantly in the study of the internal secretions, during the last decade the larvæ of the amphibians have been found an excellent material, suitable for the investigation of many problems of endocrinology. To-day the results obtained in this work seem to form a solid mass of trustworthy evidence, from which may be derived not only valuable information as to the mechanism of growth and development in amphibians, but also important knowledge as to the functions of certain endocrine glands. In the field of internal secretion, these experiments have attracted increasing interest from the beginning. It is evident at present that further clarification of many of the more important problems of internal secretion will come from the work on amphibians, as it can be and has been carried on with methods far superior to those available in the work on higher vertebrates.

Before entering into details the most prominent facts as revealed in the amphibian experiments may be pointed out. In the control of growth and development of the amphibian organism, the thyroid and pituitary glands play the most important rôles. The thymus is not concerned with the growth and development of amphibian larvæ. The functions of the thyroid and hypophysis glands, as far as they are revealed in the processes of

growth and development, exhibit a remarkable resemblance, and the secretions of these two glands can replace each other to some degree, but for the most part are specific.

Among those who have worked out these facts, J. F. Gudernatsch, Leo Adler, Bennet M. Allen and his pupil W. W. Swingle, E. R. Hoskins and M. M. Hoskins, and P. E. Smith deserve the greatest credit. Since much of their success is due to the extirpation of the endocrine glands in early embryonic stages, we should mention here also the names of three investigators, namely Gustav Born, Herman Braus and Ross G. Harrison, who have elaborated the delicate technique employed in the extirpation experiments and thus have made possible the progress which has been derived from them.

We will begin with the thyroid mechanism, as it has been studied more thoroughly than other glands, and in fact seems to be the chief factor in the control of growth and development. Its study in the amphibians as well as the entire work on amphibians was initiated by the well-known experiments on thyroid-feeding to tadpoles as carried out by Gudernatsch (1).

If tadpoles are fed fresh thyroid gland or are kept in water to which minute amounts of thyroid extract are added, a remarkable acceleration of development takes place. This development is the more conspicuous as it may occur with complete absence of growth. In tadpoles it is characterized especially by the sudden development of the fore limbs, by the atrophy of the tail, a sudden protrusion of the eye-balls (2), by the rapid shortening of the spiral gut, by the precocious atrophy of the organs of the larval mouth, which are replaced by the frog mouth (3), and by precocious ossification (4). These experiments have been repeated with the larvæ of salamanders, in which the precocious occurrences of the first moult, the atrophy of the gills and absorption of the fin of the tail, are most conspicuous effects of the thyroid application (5).

The rapidity with which these processes may take place is one of the most remarkable features of the thyroid effect.   Two normal larvæ of the species *A. opacum,* for instance, metamorphosed at an age of 86 days and measured 60 mm. at this time.   Six other larvæ of the same brood were placed in an emulsion of iodothyrine at an age of 35 days, at which time they measured 30 mm. on the average.   One week later, at an age of only 42 to 43 days and a size of 24 mm., all had metamorphosed. Moreover, 5 days after metamorphosis, *i.e.,* at an age of 47 days in one animal which was examined in sections, the visceral skeleton had undergone all those complicated changes through which the gill arches of the larvæ develop into the hyoid apparatus of the adult.   The effect of the thyroid hormone is quantitative; the acceleration of the amphibian metamorphosis increases with increasing concentration of the thyroid emulsion, as shown in Table I.

TABLE I

QUANTITATIVE EFFECT OF THE THYROID HORMONE IN THE ACCELERATION OF THE METAMORPHOSIS OF *Ambystoma maculatum*

Quantity of Iodothyrine	Age at Metamorphosis		
	Control	Iodothyrine	Difference
0.1 gm. iodothyrine in 1,000 c.c. of water	101 days	33 days	67 per cent.
0.01 gm. iodothyrine in 1,000 c.c. of water.............................	80 "	58 "	28 " "

In one experiment the larvæ of *Ambystoma maculatum* were kept in water, to which 0.1 gm. of iodothyrine per 1,000 c.c. of water had been added; in another experiment only 0.01 gm. of iodothyrine ˙was added to 1,000 c.c. of water.   In the first experiment all larvæ metamorphosed 13 days after the first application of iodothyrine; in the second experiment metamorphosis took place 39 days (on the average) after the first application of iodothyrine. The difference between the normal time of metamorphosis and the time of metamorphosis of the experimental larvæ was 67 per cent. in the first experiment and 28 per cent. in the second.

It is remarkable that the administration of the same amount of iodothyrine causes metamorphosis of salamander larvæ of different species in nearly the same interval of time. Thus, 0.1 gm. iodothyrine per 1,000 c.c. of water caused metamorphosis of *A. opacum* larvæ in 7 days, of *A. maculatum* larvæ in 13 days and of *A. tigrinum* larvæ in 13 days. The time required to induce metamorphosis in thyroid-fed tadpoles decreases with increasing age of the tadpoles. Gudernatsch (1) found that thyroid feeding caused metamorphosis in 20 days if tadpoles of a certain age were employed, in 6 days, if tadpoles 7 days older than the first lot were employed, and in only 4 days if the tadpoles were 14 days older than the first lot.

As pointed out above, the larvæ, when fed thyroid substance, may undergo the most remarkable development, although no growth may take place. This seems to be of great importance in many ways. In all organisms development and growth, under normal conditions, proceed in a parallel way. The behavior of the thyroid-fed larvæ suggests that the reason why no development takes place without growth is the fact that, under normal circumstances, the substances which cause development of certain organs are supplied through the same reactions which control the growth of the organism. If these substances are supplied to the organism from without, development may proceed at a higher rate than growth or may proceed even in the complete absence of growth and thus the relation between growth and development may become changed as in the thyroid-fed larvæ.

The changes of the relation between growth and development furnish an important link in the chain of facts that we must know in order to understand the mechanism of the thyroid apparatus as well as that of the amphibian metamorphosis. Although under certain conditions growth may be inhibited completely upon the feeding of thyroid, this is not always the case. Both the rate of development and the rate of growth are dependent

on the quantity of thyroid substance administered to the larvæ. Up to a certain quantity, growth as well as development is accelerated; if the quantity administered is further increased, growth becomes more and more inhibited, while differentiation is increasingly accelerated. With very large doses the thyroid substance may effect even a decrease in the size and weight of the larvæ; while development of the limbs is greatly accelerated in the beginning, it finally stops and the animals die from emaciation (6).

Kendall (7) has shown that in man the thyroid hormone increases the basal metabolism in a strictly quantitative way. Determinations of metabolism have not been made in amphibians, but the behavior of the thyroid-fed tadpoles as described above indicates that the thyroid hormone also increases highly the metabolism of the coldblooded organism. If too much of the hormone is administred, metabolism is increased in such a manner that catabolism becomes higher than anabolism, since the organism no longer is capable of supplying enough food materials from outside to maintain a positive metabolic balance, and consequently the body substance itself is broken down and a decrease in size and body weight takes place. Finally even development becomes impossible. For this reason, as Lenhart (6) showed, more thyroid substance can be administered without leading to a check of development if the thyroid-fed larvæ are either kept under conditions which decrease metabolism, *i.e.,* in low temperature, or are fed on substances (carbohydrates) which can be made easily available for metabolism.

From these facts it seems evident that the amphibian metamorphosis is the result of a highly increased metabolism, or more correctly, metamorphosis seems to result if metabolism is increased in such a degree and manner that catabolism becomes higher than anabolism. The question arises whether substances or agents other than

thyroid substance can cause such an increase of metab-
olism as to bring about metamorphosis. Several experi-
ments have been carried out to answer this question. But
until thyroidectomized tadpoles have been employed in
these experiments, no definite conclusions are possible;
in larvæ possessing a normal thyroid gland it can not be
decided whether the experimental conditions employed
have caused metamorphosis by raising the metabolism
directly or merely through the intermediation of the thy-
roid by precociously releasing the thyroid hormone.
Powers as well as Barfurth has shown that a sudden
cessation of food supply results in precocious metamor-
phosis. Although this is certainly true, it does not decide
the point in question, but may mean that sudden starva-
tion may precociously release the thyroid hormone. At
any rate, starvation in itself does not cause metamorpho-
sis, but is effective only if well-fed larvæ which are ap-
proaching metamorphosis and possess a thyroid capable
already of functioning are suddenly starved. The same
criticism applies to Kaufman's (8) recent experiments,
in which an advanced neotenous larva (axolotl) of *Am-
bystoma tigrinum* was given salicylic acid, whereupon it
metamorphosed promptly. This result is extremely in-
teresting as it raises most urgently the question whether
the action of iodothyrine is specific and whether the
changes of metabolism resulting from thyroid adminis-
tration are merely quantitative or also qualitative. As
pointed out, Kaufman's experiment, however, does not
answer any of these questions.

In accord with the highly increased catabolism as
caused by the action of the thyroid hormone is the fact
that metamorphosis, in its initial stages, appears to be
more a process of profound atrophy than one of construc-
tive development, although phenomena of the latter
kind frequently accompany the degenerative processes.
Among the most conspicuous processes of destruction
are the complete atrophy of the gills and the entire vas-
cular apparatus which serves the gill circulation, a con-

siderable destruction of the cartilaginous visceral skeleton, the atrophy of the larval mouth in anurans, the reduction of the intestinal coils in anurans, the complete atrophy of the tail in anurans and the atrophy of the fin in urodelans. Not before this extensive breakdown of the larval tissues has taken place and out of the remnants of the destroyed organs the new organs of the adult develop. Particularly instructive in this regard is the development of the epithelial bodies in the larvæ of salamanders; these develop from the epithelium of the destroyed gills and in the midst of the masses of detritus which result from the destruction especially of the gill vessels.

The fact that metamorphosis can be brought about by feeding mammalian thyroid substance to the amphibian larvæ, does not of course prove that the amphibian metamorphosis, under normal circumstances, is the result of the function of the amphibian thyroid gland itself. This, however, is the case, as demonstrated especially by the work of Allen (9) and of E. R. and M. M. Hoskins (10). If in an early embryonic stage of the anuran organism the thyroid is extirpated, metamorphosis can not take place at all and the tadpoles remain permanently (as far as the observations go) in the stage of an aquatic amphibian larva. Growth likewise is ultimately interfered with, although the thyroidectomized tadpoles may grow more rapidly in the beginning and even grow larger than normal tadpoles. On the other hand, if the thyroid of metamorphic tadpoles is grafted to tadpoles which are in early larval stages, metamorphosis of the latter, up to the stage of the larvæ from which the thyroid graft was taken, is caused (11). The metamorphosis of the amphibian eye is likewise impossible if it is removed from the influence of the thyroid hormone which controls the development of the eye. If eyes of old salamander larvæ are grafted to young larvæ, the metamorphosis of the graft may be retarded by as many as 7 months and will

not take place before the eyes of the host metamorphose. On the other hand, eyes of young larvæ, if they are grafted to old larvæ, can be made to metamorphose earlier than they would under normal conditions (12).

It has been said that the thyroid substance does not actually *produce* new characters, but merely accelerates the rate of their development which is predetermined by heredity.   There can be little doubt, however, that the advance of the higher vertebrates from an aquatic stage, with open gill slits and internal or external gills, and in particular all the characters distinguishing the terrestrial amphibian from the aquatic larva, could not have developed if the thyroid apparatus had not attained, at some evolutionary stage of the amphibians, its present function.   For the benefit of those who might think that the relatively short time (about $1\frac{1}{2}$ years) of observation in Allen's and Hoskins's experiments does not justify this statement, I may refer to the Texan cave salamander, *Typhlomolge rathbuni* which illustrates in a most vivid manner the effect of the absence of the thyroid gland. This salamander never develops beyond the larval stage, retaining permanently its external gills and other larval organs.   An examination of the endocrine system of this animal was made by Emerson (13); it revealed the complete absence of the thyroid gland.   It is worth while to mention briefly another interesting condition observed in this animal, namely the almost complete lack of pigment, a condition somewhat similar to that observed by Smith and by Allen in hypophysectomized tadpoles, and the highly atrophied state of the eyes.   *Typhlomolge* is a white, blind salamander.   These latter peculiarities have been attributed frequently to the absence of light in the caves, a theory which at first seems very plausible.   It would not be surprising, however, if some day these characters should be found to be the result of endocrine disturbances.   Similar to *Typhlomolge* in all the characteristics mentioned above is a European salamander, *Proteus anguineus,* which inhabits the Austrian lime-

stone caves; nothing, however, is known about the endocrine glands of this animal.

If the thyroid substance is capable of causing the development of the characters of a terrestrial amphibian, the administration of thyroid substance should cause metamorphosis of *Proteus anguineus.* Jensen (14) subjected *Proteus* to the action of thyroid substance, but did not get any demonstrable results. Many causes may have been responsible for this failure, in particular the fact that the animals were too old when they were subjected to the thyroid feeding.

It has been known for some time that the effect of equal doses of thyroid substance on the amphibian metamorphosis is the greater, the more iodine there is contained in the thyroid gland (15). Recently, Swingle (16) has demonstrated that the feeding of common inorganic iodine to tadpoles or the keeping of the tadpoles in iodine solutions accelerates metamorphosis in the same way as does the thyroid. This effect of iodine is strictly quantitative; if there is no iodine contained in the food of the tadpoles, metamorphosis is inhibited, while with an increasing amount of iodine metamorphosis is increasingly accelerated. Moreover, the effect on the relation between growth and development is the same in iodine solutions and in thyroid feeding. Weak solutions of iodine increase not only the rate of development, but also the rate of growth, while high concentrations prevent growth. There can be no doubt that at least in the metamorphosis of tadpoles, iodine is an indispensable constituent of the thyroid hormone.

Swingle (16) found that potassium iodide and iodoform had an effect on metamorphosis similar to that of iodine, while bromine had no effect on metamorphosis and growth. Thus the effect of iodine appears to be very specific when comparison is made with so nearly related a substance as bromine.

The feeding of iodine to mammalians does not produce the same effects as the administration of thyroid sub-

stance. This fact has formed the basis for the opinion
(7) that the characteristic action of the thyroid hormone
is not directly caused by the presence of iodine in the
thyroid hormone. It seems, however, more probable that
the feeding of iodine has no effect on mammalians, be-
cause the mammalian organism, for some reasons, can
not utilize an excess of iodine. It is well known that the
mammalian thyroid gland is capable of storing large
amounts of iodine (17). If, under normal conditions,
only a definite amount of hormone could be excreted by
the thyroid gland, the feeding of excess amounts of iodine
would have no effect in the healthy individual, since every
excess of iodine would be retained and stored by the thy-
roid tissue. If in the mammalian organism the thyroid
gland should be the only organ capable of elaborating the
thyroid hormone, the feeding of iodine could have no
effect in the absence of the thyroid, or in persons whose
thyroid function is insufficient. Conditions are different
with tadpoles. Swingle (16) has shown that even in
.thyroidectomized tadpoles, iodine solutions are capable
of causing metamorphosis. Apparently the thyroid
gland is not the only organ of the tadpole which can pro-
.duce the thyroid hormone.

It should be pointed out, however, that a fundamental
difference exists between frogs and toads on the one
hand, and salamanders on the other, as regards their
reaction to iodine. Salamanders behave much like mam-
malians. Although I was able to confirm the accelerating
action of iodine at least in the development of the limbs
of the tadpoles, I have not been able to cause precocious
metamorphosis by placing salamander larvæ in iodine
solutions. Table II will illustrate this statement.

Two larvæ of the species *A. maculatum* were kept first
in a solution of 5 drops $\frac{1}{20}$ m. iodine per 1,000 c.c. water
and then, up to metamorphosis, in a 3-drops-iodine solu-
tion. No acceleration of metamorphosis took place; the
larvæ metamorphosed at an age of 122 days, while the
controls were only 101 days old when they metamor-

phosed.   It is interesting to note that while tadpoles of *Rana sylvatica* are killed by a 5-drops-iodine solution and upon a 3-drops-iodine solution respond promptly with development of the hind limbs, the larvæ of *A. maculatum* showed no other effect from a 5-drops solution than a slightly decreased food intake.   The latter circumstance may account for the longer duration of the larval period of the experimental larvæ.   Since it was believed that in this experiment the solution was too weak, 2 larvæ of the same species, after a short sojourn in a 3-drops solution, were placed in an 8-drops-iodine solution; but as Table II shows, in this experiment also metamorphosis was not accelerated by the iodine solution.   Several larvæ were fed directly crystals of iodine to make sure that the in-effectiveness of the iodine solution in salamanders was not due to a possible impermeability of the larval skin for iodine.   In one case two crystal-fed larvæ metamor-phosed at 124 days, while the controls metamorphosed at the age of 101 days.   In another experiment, in which 3 larvæ were employed, one metamorphosed at the age of 89 days, while the controls metamorphosed at 80 days. Of the two other larvæ, one did not show any signs of metamorphosis when it was killed for histological ex-amination; the other one died from an overdose of iodine, but did not show any sign of metamorphosis.

TABLE II

IODINE HAS NO EFFECT ON THE METAMORPHOSIS OF *A. maculatum*

Quantity of Iodine.	Age at Metamorphosis.		
	Normal.	Iodine Solution.	Iodine Sol. + Crystals.
5 to 3 drops 1/20 m. iodine in 1,000 c.c. water...........................	101 days	122 days	124 days
5 to 8 drops 1/20 m. iodine in 1,000 c.c. water...........................	80 "	79 "	89 "

Three old larvæ, all of the axolotl type, and one neo-tenous, of the western race of *Ambystoma tigrinum,* which were collected in the Rocky Mountain lakes last

summer, were subjected to an iodine treatment. They were placed in water containing 5 drops of a $\frac{1}{20}$ m. solution of iodine per 1,000 c.c. of water and, as they showed no reaction of any kind, this concentration was increased gradually to 8 drops and in one larva to even 13 drops of iodine (3 drops of a $\frac{1}{20}$ m. solution of iodine per 1,000 c.c. of water is enough to cause growth of the hind limbs in larvæ of *Rana sylvatica*), which is more than 0.2 c.c. of a $\frac{1}{20}$ m. solution of iodine per 1,000 c.c. of water. Although these larvæ have now been in the iodine solution for 2 months, none of them has developed any tendency towards metamorphosis, while 3 other control larvæ, among them a neotenous specimen, metamorphosed 13 days after being placed in an emulsion of 0.1 gm. of Bayer's iodothyrine per 1,000 c.c. of water. Evidently the assumption suggested by Swingle (16, III), that lack of iodine prevailing in the lakes is causing the inhibition of metamorphosis of the axolotl and other urodelans, is unwarranted. I will show presently that in the inhibition of metamorphosis and in neoteny of axolotls and probably certain European urodelans we are confronted with an entirely new phase of internal secretion, namely with the differential action of temperature upon the development of various components of the endocrine system.

In a former article (20) I suggested that the inhibition of metamorphosis in thymus-fed amphibian larvæ may be caused by lack of iodine in the thymus. Swingle (16, III) has accepted and unfortunately repeated, without further testing, this suggestion. But recent experiments show that this view must be abandoned, since addition of iodine to a pure thymus diet does not enable the salamander larvæ either to grow or to metamorphose. Similarly the retardation of growth and metamorphosis of salamander larvæ kept on a pure diet of posterior lobe of hypophysis remains unaffected if iodine is added to the water.

The iodine requirement of salamanders must be extremely slight, since anterior lobe of hypophysis, a nearly

iodine-free diet, does not in any way retard growth or metamorphosis.

There are several species of salamanders (*Autodax lugubris, Autodax iecanus*) whose young do not emerge from the eggs before metamorphosis is completed. Although the larvæ of these species have no opportunity to obtain iodine from outside, these cases do not prove, of course, anything against the importance of iodine in the amphibian metamorphosis; very likely the eggs of *Autodax* contain enough iodine to permit metamorphosis of the larvæ within the egg.

Still another difference between anurans and salamanders has made itself apparent in this work. While in tadpoles, of at least certain anuran species, the development of the legs is, in some as yet unknown way, distinctly under the control of the thyroid, the leg-development in salamanders is independent of the thyroid gland. Both hind and fore limbs develop in a normal way after thyroidectomy in salamander larvæ, as shown by E. R. and M. M. Hoskins (10). Moreover, the development of the legs is not accelerated if the larvæ are kept in solutions of iodothyrine (18); this is the case even if the administration of iodothyrine is commenced soon after the eggs have been deposited. Consequently, it is very common to find that the larvæ metamorphose in the iodothyrine solution before the legs are completely developed. It is evident that in tadpoles part of the larval development is controlled by the thyroid function, since neither the hind limbs, from a certain stage on, nor the fore limbs can develop in the absence of the thyroid (9, 10, 19). Apparently the anuran thyroid gland begins to secrete already in the larval period. In salamanders the larval development seems to be highly independent of the thyroid function and it is quite probable that the salamander thyroid does not begin to function much before the first moult. This can be demonstrated in the following way (12). If eyes of old larvæ which, however, are still far enough from metamorphosis, are grafted on to young

larvæ, their metamorphosis is inhibited until the host metamorphoses. If the eye graft, however, is taken from larvæ which are near metamorphosis, such an inhibition is no longer possible. Apparently shortly before metamorphosis actually occurs, the thyroid begins to excrete, and after the circulating hormone has reached the eye metamorphosis of the eye takes place, even if the organ is transferred to an animal in which the thyroid hormone has not yet been secreted.

It is quite possible, that the late beginning of the thyroid function in salamander larvæ is one of the causes why the administration of an excess of iodine is ineffective in the metamorphosis of these amphibians. Probably the thyroid merely stores up the excess of iodine, but does not release the hormone till shortly before the first moult.

Allen (19) has recently examined the condition of the thyroid of Colorado axolotls and has found that they possess a thyroid corresponding in size, structure and colloid content to the thyroid of adult specimens of *A. tigrinum*. The thyroid of the larvæ of other salamander species likewise seems to be mature much before metamorphosis actually takes place. Allen concluded from his observations that the thyroid of salamanders begins to function at an early stage of the larvæ. The independence of the larval development of the salamander larvæ as demonstrated by the facts mentioned above shows, however, that the presence of a mature thyroid before metamorphosis must be interpreted in a different way. The most conspicuous character in the salamander metamorphosis is the fact that, although it certainly is dependent on the thyroid hormone, it does not necessarily take place in larvæ whose thyroid is mature. This can only mean that two factors are required in order to bring about the metamorphosis of salamander larvæ, namely a mature thyroid gland and a factor which releases the thyroid hormone from the follicles of the gland.

This conception, which is now supported by several

facts, is also capable of explaining the problem of neoteny of the so-called axolotl. In the course of experiments carried on during several years in the laboratory, and by inspection of the conditions prevailing in the Rocky Mountain lakes, the natural habitat of the American axolotl, I have become convinced that the neoteny of this species is due to the effect of low temperatures. We have in the amphibians an experimental material in which the relation between the development of the body and certain endocrine glands can be changed by the influence of temperature, owing to the differences of the temperature coefficients of the processes governing the development of different glands.

Although my experiments are not yet finished, they seem to permit the following conclusions in connection with my field observations:

1. The thyroid gland of salamanders undergoes a developmental change consisting of two periods, one of early development, lasting at least 63 weeks, in the course of which the thyroid becomes more and more sensitive to the action of a releasing factor (called excretor substance in my earlier work) and one of aging in the course of which the thyroid loses gradually its sensibility to the releasing factor.

2. In order to release the hormone of the thyroid gland, a particular releasing factor is required (the nature of which is entirely unknown); the quantity of this factor necessary to release the thyroid hormone depends on the sensitivity of the thyroid gland. Metamorphosis can take place only if the thyroid is sensitive and is acted upon by the proper quantity of the releasing factor.

3. The temperature coefficient for the elaboration of the releasing factor is higher than the temperature coefficients for growth and the thyroid change.

The following facts seem to warrant these assumptions:

1. Salamander larvæ, kept at an identical temperature, are nearly all of the same size when they metamorphose. Larvæ kept at low temperatures grow considerably larger

than those kept at high temperature, before they can metamorphose. This is shown in Table III (2). The temperature coefficient for the releasing factor is higher than that for growth.

TABLE III

TEMPERATURE AND SIZE OF THE METAMORPHOSING LARVÆ

Species	Series	Size in Mm.		Series
		25° C.	15 C.°	
Opacum...............	A 1916	57	67	C 1916
	XIV 1918	61	71	XVIII 1918
Tigrinum..............	S 1917	102	119	U 1917
	XLVI 1919	103	122	XLVIII 1919
Maculatum.............	LXXV 1920	52	59	LXXVII 1920

2. In very low temperatures (6° C. to 10° C.) growth is greatly slowed down and consequently the elaboration of the releasing factor must be still more retarded; yet larvæ kept at 6° C. grow less and less large before metamorphosis, when they are transferred, at increasing ages, to 15° C., as shown by an experiment lasting 63 weeks thus far. Apparently the thyroid has gone on to mature at a relatively high rate and at 63 weeks is highly sensitive and responds to smaller quantities of the releasing factor. The temperature coefficient for the thyroid change is considerably lower than those for growth and for the elaboration of the releasing factor.

3. If the thyroid can continue to develop in the absence of growth, it probably can also commence to age. Should this assumption be correct, the larvæ kept at 6° C. should finally become unable to metamorphose, if the time during which they are kept in 6° C. is sufficiently long. At present this assumption would explain why many specimens of the Colorado axolotl yield only slowly, if at all, to the influence of high temperature, and the Mexican axolotl frequently loses completely its ability to metamorphose.

4. The Colorado axolotls reach frequently a size considerably in excess of the normal maximum size of the

species as calculated from the largest known terrestrial specimens of the eastern race of this species; the Colorado axolotls are giants.   Since sexually mature specimens of the eastern race of *A. tigrinum* become giants if they are fed anterior lobe of hypophysis, the gigantism of the sexually mature axolotl could be explained if any indications of hyperpituitarism of these animals could be discovered.   On the assumption that in spite of the presence of a large thyroid the function of this organ is suppressed by the absence of the releasing factor, the overfunction of the axolotl hypophysis would be very plausible, since, as will be pointed out later on, the absence of the thyroid function causes hypertrophy of the hypophysis in amphibian larvæ.

5. The maturing of the sex organs of the axolotl is not incompatible with the assumption of an athyroidism, since, as will be discussed presently, there can be no longer any doubt that the development of the sex organs of amphibians is entirely independent of the thyroid hormone.

6. The assumption that the temperature effect can actually produce the complex phenomenon of neoteny is supported by the fact that the species *A. tigrinum* becomes neotenous only in the high and cold regions of the Rocky Mountains and the Mexican high plateau, while in the eastern part of the United States all individuals of this species metamorphose in a normal manner.   I have examined the conditions prevailing in the Rocky Mountains; to summarize briefly my observations, the axolotl is regularly found only in those lakes which are permanently exposed to low temperatures, while in the shallow lakes of lower altitudes axolotls are found only during some years and are absent during other years; apparently a succession of several years favorable in temperature conditions is required to produce the axolotl state.

7. *A. tigrinum* is the only species of North American salamanders which becomes neotenous.   This is probably

not due to differences existing between the endocrine system of the numerous species inhabiting the United States, but is explained by the fact that *A. tigrinum,* among the closely related species which I had an opportunity to test, is the only species that can stand temperatures low enough to bring about the necessary difference between the rate of the thyroid development and that of the elaboration of the releasing factor.

8. The fact that many individuals among the offspring of female specimens of the Mexican axolotl do not metamorphose even if they are brought, immediately after hatching, into conditions permitting normal metamorphosis of other salamander species, is not necessarily related to the factors discussed above, but may be due to the development of congenital thyroid disturbance in the young born by an athyroidous female.

It is, of course, well known that many structural changes, only a few of which have been studied, are required to make, out of the aquatic larvæ, the terrestrial amphibian. This is true for the anurans as well as for the urodelans. Since we know that the complex phenomenon of metamorphosis is initiated by the thyroid effect, the question arises now which of the component changes are directly caused by the action of the thyroid hormone. The fact that certain developmental processes frequently take place upon thyroid administration and therefore are a very convenient indicator in studying quantitatively the effect of thyroid substance, of iodine or of any other metamorphosis-causing agent, does not mean, in itself, that these developmental processes are caused directly by the action of the thyroid; it is possible and indeed supported by many facts, that certain of these changes will follow automatically, after the initial changes have been effected by the thyroid action. Thus, while under normal conditions, the pigmentary pattern, the legs, the tongue, the palatal teeth and the sex organs mature in salamanders during metamorphosis, they can be shown to be highly independent of

the thyroid action at least in salamanders and may, under certain conditions, occur in the absence of this action or not occur in the presence of it. I have pointed out in former articles (18) that among the many changes occurring during the salamander metamorphosis there are two which seem to be particularly closely related to the thyroid action, namely the first shedding of the skin and the reduction of the gills to mere stubs. While the succession of all the other changes enumerated above seems extremely variable, the order in which the first moult and the reduction of the gills follow each other could not be changed as yet by any of the procedures employed, inasmuch as the shedding of the skin always is followed by the atrophy of the gills. Moreover, these two phenomena have never failed to occur in the metamorphosis of the many hundreds of metamorphosing larvæ observed in the laboratory, and even in such larvæ as were forced at a very early date into precocious metamorphosis by the administration of iodothyrine and in which other changes did not occur. And furthermore, neither the first moult nor the reduction of the gills could ever be observed in larvæ, whose metamorphosis was inhibited by dietary or other means. Thus I have come to look upon the first moult and the atrophy of the gills as two of the primary components of the salamander metamorphosis. I have not enough personal experience with the larvæ of anurans, but feel encouraged through the experiences reported by other investigators to believe that in anurans these phenomena play a similarly important rôle. Certainly the first shedding of the skin seems to accompany true metamorphosis in salamanders and tadpoles as well (38), and substances other than thyroid hormone or iodine, such as the anterior lobe substance, although they may cause the limbs to grow, do not bring about atrophy of the gills in thyroidectomized tadpoles (27).

It is different with the limbs, the pigmentary pattern, the tongue, the palatal teeth and the sex organs; these five groups of organs, at least in salamanders, have

proved to be little influenced by the thyroid action. That
the development of the limbs of salamanders is not de-
pendent on the thyroid gland has been pointed out above;
here I may add that Typhlomolge is a further illustration
of this fact, as in this salamander the legs develop in a
normal manner in spite of the complete absence of the
thyroid gland. The relation of limb development and
thyroid action in tadpoles is by no means definitely
settled as yet. In tadpoles the development of the limbs
seems to be highly dependent on the action of the thyroid
gland; but attention has been called to this surprising
difference between two groups of organisms so closely
related otherwise and the suggestion has been made in a
previous article (18), that this difference as far as the
fore legs are concerned may be due merely to the fact
that in tadpoles the limbs grow beneath the skin and
consequently can not break through unless the changes
are initiated which finally lead to the shedding of the skin
and that these changes and not the thyroid action are the
primary factor in the development of the anuran fore
limbs. Whether or not this assumption is correct can
not be decided at present, but certainly deserves renewed
attention in view of recent discoveries which demonstrate
that the development of the limbs of tadpoles, at least in
certain species, is not as dependent on the thyroid secre-
tion as some investigators were inclined to think. Allen
(34), who has made prolonged observations in thyroidec-
tomized tadpoles, has recently found that not only the
hind limbs, but even the fore limbs in the thyroidectom-
ized larvæ of *Bufo* ultimately attain a size and differenti-
ation not only equal but superior to those attained in nor-
mal metamorphosing larvæ. The only difference, how-
ever, is that in the absence of the thyroid gland the fore
limbs can not break through the skin.

As to the skin pigmentation, it is well known that larvæ
in which metamorphosis has been inhibited for some
reasons may develop a nearly adult pigment pattern. In
larvæ of *A. opacum* which were fed thymus gland, and

consequently did not metamorphose, the coloration of the skin advanced to a stage very similar to that of an adult animal.   On the other hand if young larvæ of *A. opacum* are made to metamorphose precociously by means of the application of iodothyrine, metamorphosis takes place, while the color pattern remains in an early larval stage. Through the observations of Cope (35) it has become known that otherwise completely metamorphosed individuals of the species *A. tigrinum* may exhibit either a larval condition of the tongue or larval characters of the palatal teeth or larval characters in both the tongue and the palatal teeth.

In nature it is not uncommon that the sex glands of salamanders develop to complete maturity while the rest of the organism remains in a larval stage (18).   This phenomenon, known by the name of neoteny, illustrates that the sex organs can develop in the absence of the thyroid function.   The same fact has been shown in the larvæ of anurans by B. M. Allen and his coworkers.   In thyroid-fed frog larvæ, which have undergone precocious metamorphosis, the sex organs do not seem to be further developed than those of normal larvæ of the same age (3).   Moreover, if the thyroid is removed from the larvæ and metamorphosis inhibited, the sex organs develop at the same rate as in normal larvæ (21).   Hoskins (22) and Allen (21) showed that the testicle of thyroidectomized tadpoles may develop ripe spermatozoa.   These facts, however, can not be interpreted to mean that the germ plasm is independent of the somatic plasm, in the Weismannian sense.   The characteristic feature of the amphibian development is not the independence of the germ plasm from the somatic plasm, but the independence of various groups of organs from one another, due to the fact that the development of each of these groups is controlled by substances different from those controlling the other groups, and that each of these substances separately may be supplied to or withheld from the organism either by the experimenter or by conditions

not fully known as yet (18). One of these conditions is the temperature as has been pointed out above.

I will discuss briefly now the rôle of the hypophysis in the growth and development of amphibians. The most noteworthy fact seems to be the existence of a remarkable resemblance between the functions of the amphibian thyroid and hypophysis glands during the larval period. If the hypophysis gland is extirpated in early embryonic stages, the tadpoles stop to develop at a stage at which, in normal tadpoles, metamorphosis begins. Growth, too, is inhibited in the hypophysectomized tadpoles (23, 24). In a series of extremely interesting experiments Allen (25) showed that both growth and development can be restored to the hypophysectomized tadpoles, if the anterior lobe of the hypophysis of an adult frog is grafted to such larvæ. No other part of the hypophysis when grafted to the hypophysectomized tadpoles can restore growth and development, and it is certain, therefore, that it is the anterior lobe of the hypophysis which controls the growth and development of the larvæ. In tadpoles the feeding experiments as made by P. E. Smith (26) seem to corroborate the extirpation experiments. Feeding of anterior lobe to hypophysectomized tadpoles increases the rate of growth to such an extent that growth becomes as vigorous as in normal larvæ. Moreover, at the time when the normal tadpoles metamorphose and growth ceases for a time, the anterior lobe-fed hypophysectomized tadpoles continue to grow and finally attain a size in excess of that of normal larvæ. Ultimately, however, the growth of these larvæ stops and before the size is reached characteristic of the normal adult animal. The effect of feeding anterior lobe to normal larvæ is a matter still under discussion at present. Smith (26) found that normal tadpoles when fed anterior lobe grew apparently at a slightly higher rate and also metamorphosed at a slightly earlier date than normally fed tadpoles. Recently, however, Smith (36), on account of the considerable variation in the rate of growth and develop-

ment of normal larvæ, seems to be inclined to consider these differences as being of no significance. Certainly it is of no small importance that normal and hypophysectomized larvæ react so differently to a diet of anterior lobe substance; apparently part of the active principle of the anterior lobe introduced, by the diet, into the organism is made ineffective in the presence of a normal hypophysis. Not yet completed experiments on salamander larvæ seem to suggest that the larval growth of salamanders at least can not be affected by feeding anterior lobe of hypophysis; this may be due either to a destruction of the active principle in the digestive tract or to some peculiarity in the metabolism of the salamander larvæ, and is of particular interest with regard to the fact that the adult salamanders react very markedly to an anterior lobe diet, as will be discussed presently.

One of the most pertinent and yet most difficult problems of endocrinology is presented by the existence of interrelations and interactions between the various endocrine glands. There can be no doubt that in tadpoles such an interrelation exists between the hypophysis and thyroid glands. Thus Rogers (31) and later on Hoskins and Hoskins (22) found that upon thyroidectomy performed in early embryonic stages of anurans the anterior lobe of the hypophysis shows a tendency towards hypertrophy. On the other hand if the buccal anlage of the hypophysis is removed, the thyroid soon ceases to grow and to differentiate and finally presents a state of hypoplasia, as shown by Allen (30) and by Smith (36). Since the effects of the extirpation of either of these glands on general body growth and on development are quite similar and since the behavior of each of these glands after the extirpation of the other one demonstrates the existence of an interrelation between them, the question might well be asked, if the function of each of these glands can not be replaced by the hormone of the other one of them. Although this question can not be satisfactorily answered thus far, it seems highly probable that these hormones

are strictly specific in as much as neither of them can re-
place the function of the missing one. To quoting the in-
hibition of metamorphosis and growth following hypoph-
ysectomy as proof in favor of this view one could ob-
ject that in this particular case the thyroid can not effect
metamorphosis and growth merely on account of its
atrophic condition. Smith (36), however, found, that in
certain cases of partial hypophysectomy the thyroid re-
mains completely unaffected and yet no metamorphosis
takes place; only if the remaining fragment of the epi-
thelial hypophysis grows large enough to come in contact
with the neural hypophysis, metamorphosis can be ef-
fected. For this reason Smith takes the view that the
function of the hypophysis is indispensable in metamor-
phosis and that the secretion necessary for this purpose
can only be elaborated, if epithelial and neural hypoph-
ysis are in contact with each other. That neither the
anterior nor the posterior lobe of the hypophysis contains
the substance necessary for metamorphosis and that this
substance can be produced only in the body itself, requir-
ing for its elaboration the contact between neural and
buccal hypophysis, seems much supported by the fact
that, although growth may be maintained up to a certain
size, by feeding anterior lobe to hypophysectomized
tadpoles, metamorphosis can not be effected in such tad-
poles by feeding either anterior or posterior lobe. As
to the possibility of replacing the function of the anterior
lobe substance by introducing into the organism thyroid
hormone or iodine, Allen (28) fed iodine to hypophysec-
tomized tadpoles and obtained some, but not all of the
changes induced by iodine in normal and thyroidectom-
ized larvæ and seemed to be tardily inclined to the view
that the lack of the hypophsis could be compensated for
by feeding iodine. Smith, however, in his last publica-
tion (36), claims that neither thyroxin nor thyroid gland
itself causes metamorphosis, when fed to pituitaryless
tadpoles. Quite similar are the results of feeding hypoph-
ysis to thyroidectomized larvæ. Hoskins and Hoskins
(27) were able to cause growth of limbs and emaeiation

by feeding anterior lobe substance to thyroidectomized tadpoles, but could not obtain complete metamorphosis; especially the atrophy of the tail and of the gills could not be enforced.   Similarly Allen (37) points out that feeding anterior lobe of cattle does not result in metamorphosis of thyroidectomized tadpoles.

If taken together, all these results seem to indicate that although certain resemblances exist between the hormones of the thyroid and the hypophysis glands, they are nevertheless specific and can not replace each other as regards at least certain functions.

As pointed out above, the metamorphosed salamanders react on anterior lobe feeding quite differently from the larvæ.   Such differences in the reaction upon the same principle in different stages have been observed quite frequently and are apt to throw an important light on the nature of the chemical reactions involved in growth and development of different stages.   The salamander larvæ show no appreciable effect from an anterior lobe diet, whether the anterior lobe be fed alone or in small quantities added to normal food.   If metamorphosed salamanders of the species *A. opacum* or *A. tigrinum* are fed anterior lobe, the rate of growth becomes almost immediately accelerated and growth continues after the animals have reached the specific maximum size of the species; they become giants.   The latter result must be attributed to the action of a specific growth promoting hormone contained in the anterior lobe (32).

The thymus gland apparently has no effect on growth and development, although it has been believed that it contains specific growth-promoting and development-retarding substances.   It is true that in larvæ which are fed on thymus only, growth as well as metamorphosis are inhibited.   The inhibition of metamorphosis, however, is due to the fact that in the absence of growth the releasing factor of the thyroid can not form, as has been mentioned above.   Moreover, the inhibition of growth is not caused by specific hormones of the thymus, but is merely a deficiency phenomenon.   The more normal food

there is added to the thymus, the less marked does the inhibition of growth become; small amounts of thymus added to a normal diet have no effect (33). It is unknown at present which of the food substances necessary for growth are missing, although it is certain that the deficiency of the thymus is not caused by a deficiency in iodine, calcium, sodium or potassium. Many other glands, such as the spleen, prescapular lymph-gland, parathyroids, and posterior lobe of the hypophysis are more or less deficient in the growth of salamander larvæ.

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CONTRIBUTION TO THE KNOWLEDGE OF THE NUDIBRANCHIATE MOLLUSK, MELIBE LEONINA (GOULD)*

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INTRODUCTION

THE writer's attention was drawn to this rather unusual type of molluskan life (*Melibe leonina*) in observing the living animals at the Puget Sound Biological Station, located at Friday Harbor, Washington, during the summer of 1914, and inasmuch as little was known concerning the species, an effort was made to assemble such data as might be of interest relative to its habits and development. The results of this study are presented in the following pages.

It was originally intended to publish these in connection with work on the morphology of the species, now under preparation, but owing to the extent of the morphological data and the crowded condition of the morphological journals it seems best to have this other matter appear separately.

TAXONOMY

The genus *Melibe* (Rang), together with *Tethys* (Linné), constitutes the family *Tethymelibidæ*, which forms one of the numerous groups of family rank included in the sub-order *Nudibranchiata* of the opisthobranchiate *Mollusca*. The type of the genus *Melibe* was discovered at the Cape of Good Hope and described by Rang in 1829. Since that time eleven species have been added by various

authors. Gould, 1852, described *Melibe leonina,* the species upon which this paper is based, from Puget Sound, founding for it the genus *Chioræra* now merged in *Melibe.* Bergh, in a series of papers between 1863 and 1907 revolutionized the classification of the *Nudibranchiata.* He divided the nudibranchs into two sections: the *Klado-hepatica* and *Holohepatica porostomata.* Although his work was primarily systematic, it was based on morphological studies. He added six species to the genus *Melibe,* including *M. pellucida,* from the coast of Washington near the mouth of the Columbia River. His description indicates such a close similarity to *M. leonina* that it may be questioned whether this species is entitled to specific rank; material from the type locality may be necessary to settle this point. The more recent work on taxonomy by Sir Charles Eliot, 1910, modifies Bergh's work to some extent.

No detailed study has been made of *M. leonina* previous to the present work and our knowledge of the species rested largely with the brief description and figure presented by Gould.

DISTRIBUTION

Melibe, as far as known, is restricted to the Pacific and Indian Oceans, and to the South Seas. On the eastern
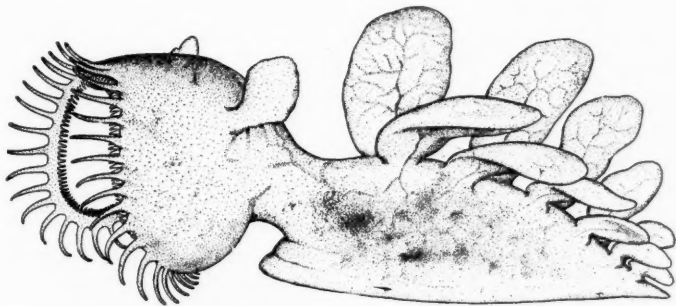

FIG. 1.   *Melibe (Chioraera) leonina* Gould. From a drawing by Mr. Bert Elliot, private artist to Professor Trevor Kincaid, University of Washington. Slightly changed. × 1/3.
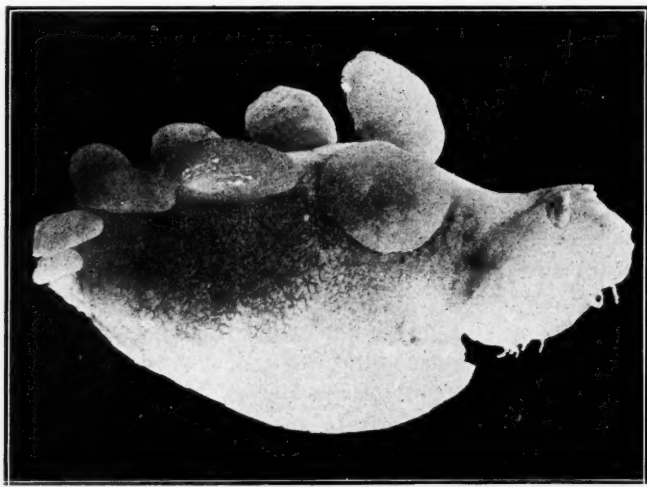
FIG. 2.   Photograph, by author, of preserved specimen of *M. leonina* showing pro-
fuse branching of hepatic system in the body-wall; the hood is contracted
into a knob as when the animal swallows.   Slightly reduced.

side of the Pacific, two species have been described,
M. leonina Gould, 1852, and *M. pellucida* Bergh, 1904.
On the western side of the Pacific, various species have
been found by Bergh from the Japanese Island to the
Straits Settlements.   One species has been described by
Angas, 1864, from Australia; one from the Pacific by De
Filippi, 1868; one from the Sandwich Islands by Pease,
1860; and several species from the Indian Ocean by Rang,
1829, Alder and Hancock, 1864, and Eliot, 1902.   So far no
species of this genus seems to have been found on the
coasts of the Atlantic Ocean.   In these parts of the world,
and in the Mediterranean and Caribbean Seas, it seems,
according to works of Bergh, 1877, 1890*a*, Locard, 1885,
Viguier, 1898, and Eliot, 1910, to be replaced by *Tethys*
Linné.   Eliot 1910 (pp. 12–13), fails to record *Melibe*
among the American Pacific fauna.   Neither *Tethys* nor
Melibe seems to occur in the northern waters.   All the
Melibidæ recorded by Agassiz, 1852, G. O. Sars, 1878,
Johnston, 1838 (*Melibea*), are, in fact, not *Melibe*, but

Doto. Von Marten's (1879) *Melibea* does not seem to come under either type, although from this author's description certain characteristics are in common with that of *Melibe.* For some time there was considerable confusion in regard to these genera. Bergh, 1863, and again in 1871, makes this clear when he writes:

Das Geschlecht Doto wurde von Oken (1815) auf der Gemlin'schen *Doris coronata* (Bomme) aufgestellt. Jahre nachher (1829) bildete Rang (Man., p. 129, pl. 3, f. 3) eine neue Geschlechtsform Melibe ab, dessen Typus eine Nacktschnecke war, die er im Meere des Vorgebirges der guten Hoffnung (Cap) am schwimmenden Meeresgrase fand. Das Rang'sche Geschlecht, das von späteren Verfassern gewöhnlich Melibaea genannt ist, ist am meisten mit dem Oken'schen Doto identificirt worden, in der Art, dass alle bisher bekannten Melibaeen—eben mit Ausnahme der "Melibe" rosea von Rang—der Doto-Gruppe gehören. Die Melibaea, autt. sind in der That mit den Doto'en identisch und mussen diesen Namen bekommen. Die Meliben Rangs dagegen werden, wie früher von mir hervorgehoben (Schiödte Naturh. Tidskr., 3 R, I, 3, 1863, p. 480), einen ganz verschiedenen Geschlechstypus bilden, den ich als mit den Chioraeren von Gould verwändt betrachtete, der aber einer späteren Mittheilung von Alder und Hancock (1864) zufolge den Scyllaeen näher (?) käme.

The genus *Tethys* Linné, 1758, the nearest relative of *Melibe* Rang, 1829, was also confused with other forms, *e.g., Aplysia.* Pilsbry, 1895, has, however, cleared up this point. He shows that the various specific names attached to *Tethys,* such as *fimbria, fimbriata,* and *leporina,* are to be considered synonyms. Bergh, 1880*a,* and Krause, 1885, do not record *Melibe* in their nudibranchiate collections from the north Pacific. Likewise, investigators in the north Atlantic, on both sides of the ocean, including a number of explorers and independent workers such as Alder and Hancock, 1842 and 1845; Meyer and Möbius, 1865; M. Sars, 1870; Aurivillius, 1885; Garstang, 1890; Krause, 1895; Ohdner, 1907; Walton, 1907; Théel, 1908; and Johnson, 1915, do not record *Tethys* or *Melibe* in their collections. These species are, therefore, restricted to the warmer seas.

When the Puget Sound Biological Station[1] was estab-

[2] This station was first known as the Puget Sound Marine Station; its founder, Professor Trevor Kincaid, remained its director until 1914.

lished in the San Juan Archipelago in 1904, it was found that *Melibe leonina* was not uncommon in the vicinity, although like many pelagic organisms its abundance was subject to great fluctuations.   In the summer of 1912 it was particularly abundant, great numbers appearing among the fronds of *Nereocystis* drifting past the floating dock in front of the station.   At this time Prof. H. L. Osterud, of the University of Washington, gathered and preserved a considerable number of specimens.   The largest specimen obtained was six cm. in length, the time of collecting being the latter part of July.   During the season of 1913 very few were seen.   In 1914, the writer found several specimens of large size, 8 to 13 cm. in length.   These were taken among the floating eel-grass, *Zostera marina*.   In the summer of 1915 only two specimens were found.   It appears that the genus *Tethys* is of spasmodic recurrence in the Mediterranean (Viguier, 1898).   The appearance of *Melibe* does not seem to be determined by any particular season, as Prof. Osterud found specimens spawning when visiting the Biological Station early in March of 1916.   The period of existence of this nudibranch must be more than one year, or one season, judged from the sexual condition in this species which has shown maturity in individuals ranging in size from two to fourteen centimeters.   Alder and Hancock (1845), p. 24, think the period of existence for a large number of species ''not much exceeding one year.''

Ecology

There is a striking similarity between the *Klado-hepaticæ*, not only between the members of any given family, but also between members of different families within the section.   The former is well illustrated by *Tethys* and *Melibe,* members of the family Tethymelibidæ. This similarity is not only morphological, but equally true as to manner of living and general behavior, for instance, the method of swimming.   *M. leonina* may crawl on the leaves of the eel-grass; or it may float with

the back up, the hood having air under it, or the papillæ
serving as floats. In the latter case *Melibe* alternately
bends laterally the anterior end to angles of 45 degrees.
During these alternating bendings of the body the move-
ments are swiftest when the body is relaxed from the
45-degree bend. By this method it conveys itself slowly
through the water. However, on the surface tension of
the water or on the eel-grass, the movements are caused
by the ciliary action of the ventral surface of the foot,
because progressive movements occur without visible
bodily contortion. *Tethys leporina* Linn. moves through
the water by similar means (Bergh, 1877 and 1883). In
its case, however, the large veil, as well as the lateral
bendings of the body, plays an important part. Gould,
1852, in his original description of *Melibe leonina,* says:

This animal swims by lateral flexions of the body, the foot being then
folded; and when crawling it is able to flex its enormous head laterally
with considerable force (p. 310).

Scyllæa pelagica Collingwood, 1879, swims very much
like *M. leonina* and *T. fimbria;* and Garstang, 1890, men-
tions that *Lomanotus* Vérany, swims "vigorously through
the water in the dish . . . by lashing the body from side
to side." But neither Gould, Bergh, Collingwood, nor
Garstang mentions ciliary action as a factor in locomo-
tion.

Collingwood, 1879, says in part:

Considerable numbers of this pelagic species were found upon *Sar-
gassum bacciferum,* floating in Lat. 25 N., Long. 37 W., most species
of weed having one or more specimens. The animals were in constant
movements of contracting and writhing. In the water they swam freely,
moving the head and tail from side to side alternatingly, so as nearly
to touch one another; and when thus swimming were always, owing to
the weight of the papillary prolongations and tentacles, back downward,
and bore grotesque resemblance to a four-legged animal with ears, such
as a Skye terrier.

Another similarity between *Scyllæa* and *M. leonina* is
the manner of dropping from the surface to deeper
water. In the case of *leonina* this is a sort of death
feigning. *S. pelagica* may be found at the surface or in

deeper water.   Collingwood mentions it as assuming
certain aspects when it falls through the water to a con-
siderable depth, where it is frequently found. *M. leonina*
possesses the same habit.   In so doing the muscles of
leonina are absolutely relaxed and the animal appears
dead.   The means by which the animal gets to the sur-
face are not known, unless it be by its general mode of
swimming.   One striking difference between these two
forms is: *M. leonina* may swim with the back upward,
"the weight of the papillary prolongations," important
in *S. pelagica,* being apparently of no account.

The eel-grass offers an excellent feeding ground for
Melibe. Here the water must not only be calm, but may
abound in small Crustacea. *Zostera,* which grows in
large beds in the bays near the Biological Station at
Friday Harbor, offers also a suitable assembling place
for *Melibe* where it may pair and lay its eggs. At low-tide
the eel-grass floats on the surface of the water and leaves
many inclosures of open water.   In these open spaces
M. leonina collect and copulate, as was observed in the
summer of 1914.   In such spaces, as described, a con-
siderable number of *Melibe* had collected, and some of
them were copulating, being united head to head, the foot
of one mate facing the surface.   The excellent condition
of the water offered an ample opportunity to study the
mode of swimming and the manner of feeding.   The
former has partly been described above, and will further
be discussed under the topic on observations in the labo-
ratory; the latter corresponds to Eliot's description
(1902) of *M. fimbriata* Ald. and Hanc.   *M. leonina* is not
so definite in its movements during its feeding as is
M. fimbriata, yet some similar method of feeding is pur-
sued.   Both species have a large hood.   In the case of
leonina the hood is extended very widely (Fig. 1), when
the animal is searching for food, and is periodically con-
tracted into a knob (Fig. 2), when food is obtained.
When the hood is open, *leonina* tosses it sideways, hold-
ing it in direct position for the capture of small horizon-

tally swimming crustaceans.   Eliot, 1902, says of *M. fimbriata:*

> In spite of its want of jaws, *Melibe fimbriata* is a most voracious animal, and I more than once found in the stomach which I examined limbs of Crustacea more than an inch long. . . . The movements of the animal are rapid and energetic, whether it crawls or swims. It can float on the surface, foot uppermost (p. 70).

Melibe leonina is actively predaceous also; its gizzard has been found completely filled (Agersborg, 1919) with minute Copepoda, Amphipoda, and larger and smaller Isopoda, until the gizzard would bulge out into almost a perfect sphere; ordinarily the gizzard has only a partial enlargement; its normal size is a little larger than that of the proventriculus, and the anterior part of the intestine.

Daugherty and Daugherty, 1912 and 1917 (p. 83), refer to nudibranchs as vegetable feeders; having mentioned *Eolis* and *Pleurophyllida,* they say:

> These soft naked sea-slugs live in shallow water near the shore, crawling about and feeding upon the sea-weeds.

However, only a few of the rarer species are phytivorous; the majority are carnivorous, a fact which is recognized by the authorities on nudibranchiate *Mollusca.*   Thus Bergh, in most of his descriptions of nudibranchiate fauna, reports in favor of animal diet: 1880*a* (*Akiodoris lutescens*) p. 56, (*Lamellidoris bilamellata*) p. 64, (*L. luptricina,Acanthodoris pilosa*) p. 101,(*Triopha modesta*) p. 116; the food of these forms consisted of "indeterminable animal matter, mixed with some diatomaceæ, . . . and with some Polytholamia, . . . with larger and smaller pieces of small Crustacea, . . . and a little indeterminable worm, of the length of 2.0 mm., . . . spongiary masses and different Radiolariæ of a diameter of 0.09 mm." In regard to *Tritonia reticulata,* the same author, 1881, says:

> Die Tritoniaden sind Raubthieren und scheinen sich hauptsachlich von Alcyonien und ähnlichen Thierformen zu ernähren.

Again, 1883, referring to *Tethys leporina,* he says:

Tethys ist ein Raubthier und sein Nahrung besteht namentlich aus kleinen Ophiuren, deren Reste oft ganz den Magen erfüllen.

1890*a*:

Der Magen und der Darmen von Nahrung vollgeslofft; dieselbe bestand aus Massen von kleinen Decapoden, mit Bruchstücken von kleineren Gasteropod Schalen und Sandkörnern vermischt (p. 158).

1894 (*Dendronotus robustus*):

In der verdauungshöle unbestimmbare thierische Masse, mit Diatomeen vermischt (p. 144).

And finally, describing the food of *Melibe rosea*, 1907, he says:

The contents of the alimentary cavity (specimen 1.5 cm.–3.8 cm.) were animal matter with remains of small Hydroids (p. 98).

Alder and Hancock, 1845, p. 23, say:

But, though so patient and long-suffering in the endurance of hunger, these little animals are very voracious. The greater number of them are carnivorous; living principally upon zoophytes and sponges. The *Alcyonium digitatum* is a favorite food with the *Tritoniæ;* and the *Actiniæ* and *Lucenariæ* often fall prey to the attacks of the *Eolides*. These latter, indeed, do not scruple occasionally to devour the weaker among their own brethren, as we have recorded elsewhere. Sir J. G. Dalyell states that his *Eolis histrix* (Drummondi) 'fed voraciously on mussel, and on common periwinkle, whereof large portions were swallowed entire'; and he thinks that *Goniodoris nodosa* feeds upon *Ascidia papilla* (*Cynthia rustica*), to which he attributes the reddish colour observed in the viscera. This colour, however, is caused by the liver and ovary. We have taken from the stomach of *Eolis papillosa* minute specimens of the common mussel, and a small *Terebra* from that of Tethys. The more common food of the tribe, however, is the flexible zoophytes. Until lately the Dorides have been considered vegetable feeders, but this would appear not to be the case. *Doris tuberculata* feeds upon common encrusting sponges (*Halichondria panicea*), and sponges and zoophytes seem to constitute the food of most of the others. A few of the gregarious Nudibranchs, such as *Polycera quadrilineata, Hermæa dendritica,* and *Alderia modesta,* which congregate on marine algae, appear to be phytivorous; but *Eolis despecta,* and *E. exigua* though not unfrequently gregarious on the fronds of *Laminaria digitata,* are only found on those parts of the plants that are covered with the parasitic zoophytes, *Laomedea geniculata* and *L. gelatinosa,* on which they feed and deposit their spawn.

Meyer and Möbius, 1865, are of the opinion that it is

rather difficult to determine whether Nudibranchs are carnivorous or phytivorous, that is, that the food of Nudibranchs is very variable. In part they say of *Elysia viridis* Montagu, p. 10:

Sie nährt sich wahrscheinlich von Pflanzen.

Page 23:

Eolis alba frisst, wie die anderen Kieler Arten ihrer Gattung, thierische Stoffe.

Page 31 (*Eolis papillosa*):

Ihre Nahrung sind Thierstoffe; besonders liebt sie Actinien. Kleinere-Exemplare der *Actinia plumosa* greift sie am Fussrande an, und frisst ein halbmondförmiges Loch hinein, das sie immer mehr vergrössert.

This is also the opinion of Hecht, 1895, p. 621:

Il n'est donc pas possible d'établir à ce point de vue une division bien tranchée. On peut dire seulement, que les familles les plus franchement herbivores sont les Hermaeidae et les Elysiidae, et en général les Ascoglosses qui, comme Ihering et d'autres l'ont remarqué, ont une masse buccale disposée pour exercer une succion. . . .

Page 622:

Les Eolidiens sont tous franchement carnivores et présentent, parmi les grandes espèces, quelques types d'une voracité extraordinaire. *Eolis coronata* . . . devore des *Elysia viridis;* à l'autopsie j'ai trouvé des radulas dans son tube digestif. Les petites espèces *Eolis despecta, E. exiqua, E. olivacea* peu faites pour de grands déplacements, vivent à demeure, comme je l'ai dit plus haut, sur des colonies d'Hydroides.

Page 223:

Calma glaucoïdes, qui, pendant une période de sa vie tout ou moins, se nourrit, je l'ai dit, d'embryons de Poissons. Le régime des Doridiens est moins uniforme; certains genres sont probablement herbivores. Plusieurs espèces de Doris se nourrissent d'Eponges calcaires dont on retrouve les spicules dans les excreta. . . . Plusieurs espèces de Goniodoris se nourissent de Bryozoaires. Il est probable que *Polycera quadrilineata* mange des Algues. Il faut signaler ici les observations de Prouho, sur la façon particulière dont *Idalia elegans* se nourrit de certaines Ascidies. Quant aux Ascoglosses, j'ai indiqué plus haut que *Hermaea dentritica* dévore les couches superficielles des Codium tomentosum, qu'elle réduit à l'état d'un petit moignon verdâtre. *Elysia viridis* se nourrit aussi de Codium tomentosum, mais sans marquer de préférence pour telle ou telle région; j'ai du reste observé qu'elle s'accommode aussi d'autres Algues.

Jeffreys, 1869, contributes to this subject and says:

Although most of the order are zoophagous, *Limapontia* and others of a simpler kind feed on seaweeds.

And von Ihering, 1876, p. 37, referring to *Tethys* states:

Das der Magen eines so gefrässigen, jeder Bewaffnung des Mundes baaren Raubthieres wie *Tethys* eines solchen Schutzes ganz besonders bedarf, wird sofort verständlich, wenn man den Mageninhalt desselben kennen lernt. Ich fand denselben ausser aus Tangstücken bestehen in zahlreichen Crustaceen, kleinen Echinodermen und mehrmals auch kleinen Fischen, von denen einer 4 cm. lang war. Dasselbe Thier enthielt noch die Otalithen eines andren Fisches, welche diejenigen des eben-bezeichneten um das Doppelte übertrafen.

Melibe fimbriata Ald. and Hanc., is, according to Eliot, 1902,

in spite of its want of jaws, a most voracious animal.

This same author says he more than once found in the stomach he examined limbs of Crustacea more than an inch long. And, in 1910:

Thus the red British Dorids *Rostanza coccinea* and *D. flammea* eat red sponges, such as *Microciona atrasanguinea* (p. 5).

In fact, this author thinks that most Dorids feed on sponges (Eliot, 1910, p. 39). Step, 1901, referring to the crowned sea-nymph, *Doto coronata,* says it feeds upon Hydroids (*Sertularia* and *Plumularia*) and Corrallines (*Antennularia antennina*). The marble slug, *Lomanotus marmoratus,* feeds upon corrallines which it closely resembles in color and ornamentation. Eolis feeds upon anemones, *Sagertia, Lucernaria;* Sea-mats, *Tubularia,* various sponges and *Obelia.* The crimson Hermæa (Ascoglossa), *Hermæa bifida,* feeds on small crimson weeds (*Bryopsis, Codium, Enteromorpha* and *Ulva*). Vayssiere, 1901, p. 84, referring to *Tethys fimbria,* Bohascht, Delle Chiaje (Synon. *T. leporina* Linné, Cuvier), writes:

Dans l'intérieur du premier renflement stomacal (jabot), je trouvais d'ordinaire une grande quantité de filaments fibreux de Zostères; ces mollusques doivent en aspirant avec leur trompe, absorber des débris de

ces végétaux et dissocier leurs fibres par les contractions répétées des parois musculaires de cette poche.

Page 85:

Au milieu de ces débris, ils trouvent de petits crustacés (*Entomostracés, Amphipodes, Isopodes,* jeunes *Décapodes brachyures*) et autres petits Invertébrés, circulant parmi les Zostères, qui doivent former la base de leur nourriture.

Thus it is seen that even the carnivorous *Tethys* may be phytivorous.   Vayssiere, 1911, p. 43, says of *Halgerda willeyi* C. Eliot, 1903:

La poche stomacale était remplie de gros débris Crayeux constitués par des fragments de madrépores et de bryozoaires que ce mollusque arrache et broie avec sa forte radula.

Finally, MacFarland, 1912, p. 530, says in regard to the Dironidæ (*Diron allolineata*):

Diatom shells and minute spicules, these made up a very small portion of the total contents.

Nudibranchs may be said to be omnivorous; as seen above, a species which is void of radula, *e.g.*, *Tethys*, may at one time be carnivorous, at another time phytivorous; likewise forms possessing radula (*Acanthodoris*) are omnivorous.   The largest number seem to be carnivorous notwithstanding; a few, the *Hermæidæ* and *Elysiidæ,* are phytivorous.

Means of Defense

Upon being first encountered, *Melibe leonina* appears brown, but when examined in the aquarium one can easily see that the brown coloring is rather superficial in comparison to the marked transparency which the body possesses.   This transparency is so great that the internal organs, such as the alimentary canal, the organs of reproduction, and the heart, can be easily seen.   The possibility of actually seeing these organs through the body wall is due to the arrangement of the muscles and the connective tissues, and because the body-fluid contained in the perivisceral cavity, between the visceral

organs and the muscles, the connective tissues, as well as
the blood, are colorless.   This characteristic is also
common to *M. pellucida* Bergh, 1904; and *M. vexillifera*
Bergh, 1880.

Upon touching the curious-looking animal it gives off
a peculiar odor.   This is rather strong, and resembles
that of oil of bergamot.   It is caused by a secretion from
small compound saccular glands lying immediately under
the ectoderm (Fig. 12).   These glands are distributed all
over in the external parts of the body: in the body wall,
the papillæ, under the ectoderm of the exterior part of
the hood, and in some cases, under the ectoderm of the
foot.   None of these glands seem to be present under the
ectoderm of the ventral side of the hood.   The extent of
distribution of the odoriferous glands seems to indicate
that they have a definite use and purpose, *e.g.,* that of
defense.

Meckelii, 1838, describes the odor exuded by *Tethys
leporina* as resembling citron, or being rather pleasant.
And Bergh, 1877, says:

Von . . . toten Thiere habe ich irgend eine Spur bemerkt, dagegen
einen nicht starken, etwas besonderen, aber nicht wesentlich unbehab-
lichen Gestank.

Hecht, 1895, discusses the various means of defense pos-
sessed by Nudibranchs.   He mentions protective colora-
tion, nematocysts, mucous glands and death feigning.
The last will be discussed presently; the first two may
only be referred to, as *M. leonina* possesses no protective
coloration, and has no nematocysts as a defensive means.
It may, however, be stated that in the aquarium, *Melibe
leonina* became even more transparent than it was when
seen in its natural environment.   This change of color
was also observed by Alder and Hancock, 1845, on a num-
ber of Nudibranchs kept in captivity: " In such cases
they generally lose a good deal of colour and become
very transparent," and that coloration is not caused by
the color of the food taken, but by the color of the liver
and gonads.   Eliot, 1910, says:

The colour of Dorids is to some extent affected by their food, though less than that of Eolids. The brightly coloured species often frequent and feed on similarly bright sponges or Ascidians, and when they do not obtain their usual food in confinement they lose their colour (p. 5).

An increased transparency when kept in an aquarium, *e.g.,* a glass-jar, may be designated adaptive coloration. The odorous substance that the animal exudes when touched by an enemy is its main protection. However, Step, 1901, records some very interesting facts relative to protective coloration among Nudibranchs:

Dendronotus frondosus is obviously adapted to life among sea-weeds and coralline, resembling some small red-brown sea-weeds (*Collithamnium*). It is said to be highly edible, having nothing in its flavor to displease the taste of the most fussy fish; and therefore its disguise is absolutely necessary to the species (p. 288–289).

Evidently *Melibe leonina* needs no protective coloration, having, as said above, odoriferous glands. One reason why it does not possess nematocysts is perhaps because it does not live on Hydroids, but mostly on Crustacea.

Glaser, 1903, in his discussion of the origin of nematocysts in Nudibranchs gives a historical review, including a number of citations all of which refer to the nematocysts as having been taken in with food. The food then seems to be the origin of the nematocysts in the Nudibranchs; *M. leonina,* which does not feed on hydroids, has no nematocysts in its system. The marble slug, *Lomanotus marmoratus,* according to Step, "Feeds upon Corallines which it closely resembles in colour and ornamentations"; Gamble, 1892, however, says that cnidocysts are absent.

Bergh, 1890a, mentions experiments by Krugenberg, 1880, who tried to determine the physico-chemical constituents of the odoriferous glands, as well as of the liver and blood of *Tethys fimbriata* (s. *leporina*), and says that *T. leporina* has a peculiar musk-like nauseous odor which it uses as a means of defense against its enemies. This is, without doubt, the office of the odor in the case of *M. leonina* also. The actual nature or constituents of

the substance which causes this defensive odor have not
been determined.

Another means of protection is self-mutilation, exem-
plified by *Discodoris fragilis,* that according to Eliot,
1899, throws off part or the whole of its mantle edge.
Collingwood, 1868, also records this habit of self-
mutilation of a *Doris.*

Embryology

The Egg-body (Nidosome)

Bergh, 1902, describing the egg-body of *Melibe bu-
cephala* says:

> The spawn forms a large heap of a diameter of 3.5 cm., composed of
> the innumerable windings of a dull yellow tube of a diameter 0.75 mm.
> The tube contains inside of the tough transparent covering several
> series of displaced, more or less cleft eggs.

From this description it is clear that this nidosome is
quite different from that of *M. leonina;* it seems, indeed,
strange that the egg-body of two closely related species
can differ so widely.   The external features of *M. bu-
cephala,* according to Bergh's description, are not much
different from those of *M. leonina.*   It may be a question
whether the egg-body attributed to *M. bucephala,* by
Bergh, actually belongs to this species.

The writer during the summer of 1914 found several
nidosomes among the eel-grass, but it was not known to
which animal they belonged until *Melibe leonina* was
seen to lay the same kind in the aquarium of the labora-
tory (Agersborg, 1919).   These nidosomes of transparent
mucous, or gelatinous substance, were funnel-shaped,
when suspended in the water, with the apex attached to
some solid object (Fig. 3).   The average slant-height of
these conical structures was 5 cm., with a perimeter of
about 28.2 cm. and a convex surface, therefore, of about
70.50 sq. cm.   From the adhering point of the nidosome,
dotted lines, the capsules radiated to the periphery of
the conical body.   This radiation was not so regular in

some as in others, yet there was a prevailing regularity
in this respect.   In Fig. 3, the arrangement of the cap-
sules does not represent the prevailing regularity, as it
was necessary to select an entire nidosome for photo-
graphing, most of the other having been broken.   The
capsules contained from 10 to 22 eggs (Figs. 4–6).   The
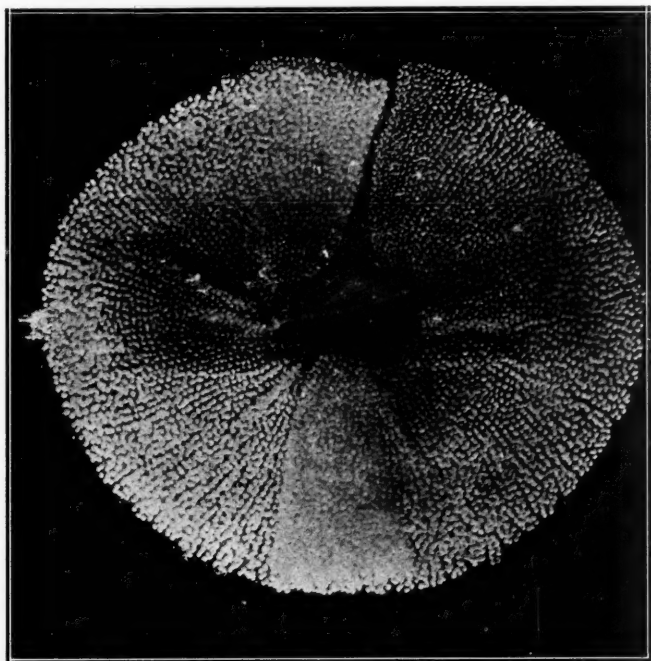actual method of deposition has not been observed, but

Fig. 3.   Photograph of a nidosome of *M. leonina,* natural size.   The dark
band at right angle to the fold is a piece of eei-grass to which the egg-body is
attached.   The many radiating white dots are egg-capsules containing from
15–22 eggs.

it is conjectured from the knowledge of the anatomy of
the animal that the capsules are imbedded in the gela-
tinous mass as the nidosome is deposited.   The mucous
gland, which consists of (1) albuminous gland, (2) nida-
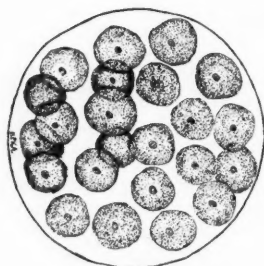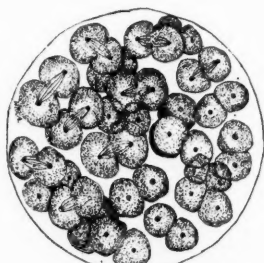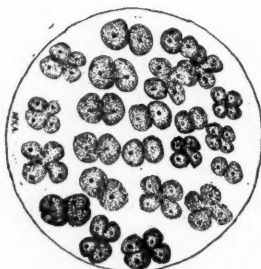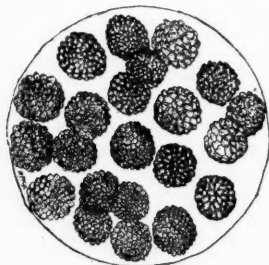mental gland (Lang, 1896), is in *Melibe* in direct connec-

FIGS. 4–7. Drawing of egg-capsules from a nidosome laid in captivity, showing a varying number of eggs. Figs. 5–6 show the eggs in two and four-cell stage, three hours after the nidosome was laid. In Fig. 7 cleavage has reached the blastula stage. The more oblong embryos are moving within the capsules.

tion with the vaginal orifice. In copulation, the penis, which is long, twisted like a screw, and of tough musculature, is inserted into the posterior genital pore of the mate, and so firm is the union that separation may not occur even though the couple be dipped from their natural abode and placed in a vessel.

Observations in the Laboratory

A study of *Melibe leonina* from an embryological standpoint, was made at the Biological Station. From the lot collected, some were preserved, others were kept alive in an aquarium. One morning, however, all save one were

dead.   Later, this one also seemed dead, and it was
thought that the water had become stale.   *Melibe* lay
absolutely motionless on the bottom, all its muscles com-
pletely relaxed, and showed signs of life only after the
water had been oxygenated for several minutes.   It is
muscular relaxation of this sort that *Melibe* assumes
when it sinks from the surface to deeper water.   After
this the writer became used to its death feigning and
needed only to oxygenate the water for it to become active
again, crawl along the bottom and side of the aquarium,
and after a while start swimming in the vessel.   Changing
of the water could not be done indefinitely, as on another
morning a nidosome was found to have been deposited
by the animal during the night.   It was a funnel-shaped,
transparent, gelatinous body adhering by the tapering
end to the side of the jar.   Viguier, 1898, when trying to
prepare a specimen of *Tethys fimbriata* for fixation, ob-
served the same phenomenon.   He, however, did not
change or oxygenate the water; he left the animal in it
for about two weeks, when he found that an egg-body had
been deposited, the eggs having ceased to divide in the
four-cell stage.   The nidosome of *T. fimbriata* is quite
different in shape from that of *M. leonina.*   In the case
of *Melibe* the eggs continued to divide until they were
transformed into larval forms, which actually turned the
whole nidosome into a vibrating mass.   The development
continued, apparently normally, until the larvæ left the
capsules, when they soon died.   That the delicate mol-
luskan young should die when coming in direct contact
with the water of the aquarium was expected, as the
renewing of the water was stopped after the nidosome
was deposited, it being thought undesirable to disturb it
too much; after the deposition of the egg-mass the water
was simply kept at constant level, and oxygenated from
day to day, so that the animal should not die.   When the
young *Melibes* were hatched the mother animal, without
being further inseminated, laid another nidosome, which
also hatched two weeks later.   The eggs of the first nido-

some developed into distinctly living creatures, moving about in the capsule on the fifth day after the setting (Fig. 7); it took two weeks for the complete development of the young.   Alder and Hancock, 1845, p. 25, say:

> The embryo matures after deposition of spawn, from a few days to a month or more, according to species; the actual time appears to be about ten days or a fortnight.

Temperature, no doubt, plays an important part in the speed of development.   Stuart, 1865, says:

> Gewöhnlich wird von den Opisthobranchiereiern angegeben, dass die Dauer der Entwickelung des Embryo ein Monat ist, in meinem Falle war sie circa zwei Monate; dabei war die vorherrschende Witterung, die für Sicilien jedenfalls eine kalte zu nennen war, gewiss von grossem Einflusse (p. 96).

The second nidosome was to some extent abnormal, compared with the first, and with those collected from the eelgrass.   It showed a variation of the number of eggs in the capsules, from one to fifteen (Figs. 8–9).   This abnormality was perhaps an indication of the decline in vitality of the mother animal.   In fact, the adult specimen had greatly decreased in size since its capture.

One difficulty was that of keeping the water at a constant density.   In order not to break the nidosome the water was only oxygenated and kept at a constant level. Each time when the eggs were examined, small portions of the nidosome were removed, and by so doing the membrane of the egg-body was broken.   This did not seem to affect the development, however.   Yet it was thought safer to keep the water at the same temperature as hitherto, than to change it daily, as the latter might cause too great physical shock.   The abnormality of the water, as said, did not affect the embryos as long as they were within the capsules of the nidosome.   Perhaps thus far in their development they were not affected by the abnormality of the water; even though the egg-body was punctured and broken in the examination of the eggs, the embryos seemed all to develop, as far as could be detected,

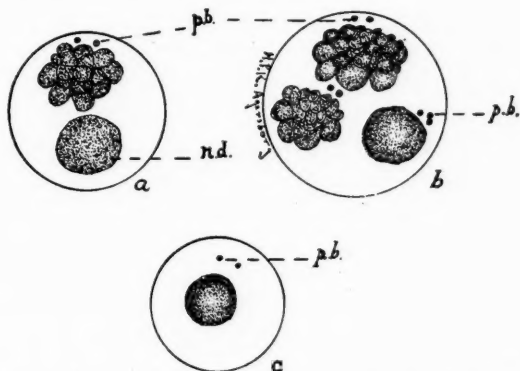and to pass through the normal developmental changes
of a typical gasteropod.

FIG. 8.   Three abnormal egg-capsules from the second nidosome deposited after
insemination; *a, nd.* no cleavage. *pb,* polar body; *b, pb.* first polar body has
divided, but no cleavage in the egg; *c,* no cleavage of egg although the polar
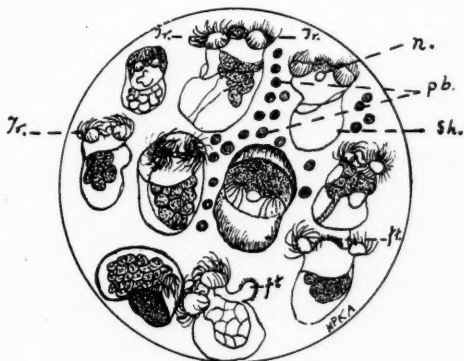bodies were given off.

FIG. 9.   An average sized capsule from the second setting after insemination;
n, pedal ganglion; *ft,* foot; *pb,* polar body; *sh,* shell; *tr,*
velum; all the embryos are in the veliger stage.

Abnormality in the second nidosome was marked by
the reduced number of eggs within the capsules and in
the early development in the eggs.   The variation of the
number of eggs in normal capsules was most marked at

the end of the egg-belt. This abnormality was much
greater in the second nidosome, in that there was a much
greater number of small capsules with only one or a few
eggs in them. There were also capsules actually without
eggs. Some eggs failed to develop; some gave off the
polar bodies and then did not advance any farther; others
did not form polar bodies. It was of great interest, in-
deed, to watch the development of the embryos in the
large capsules which in some cases contained more than
twenty eggs (Fig. 7). Within a few hours, there would
be twice or even three times as many polar bodies as eggs
(of course within the capsules), because the first polar
body sometimes divided. A detailed study of the blasto-
meres was not undertaken. Figures 4 to 6 show a few
early developmental stages, and early and late larval
stages are shown in Figs. 7 and 9. An embryonic shell
is shown in Fig. 10; veliger larvæ in Fig. 9.

When the embryos reached the gastrula stage they
swam about within the capsules. On the fifth day after
being laid the whole egg-body was practically alive with
imprisoned swimming larvæ (Fig. 9). Nine days later,
the larvæ began to leave the capsules. It was surprising
to see how rapidly the embryos advanced from day to
day, going through the trochophore and veliger stages.
During the latter stage the shell was very prominent; it
resembled the shell of *Natica russa,* in that it had a blunt
apex and short body; the posterior or tapering part of
the shell had no spiral turns; the posterior edge of the
aperture had a small indent; the edge of the aperture
of the shell was otherwise without any modifications
(Fig. 10). The animal itself did not assume the adult
shape before it lost the shell, but when it left the capsule
it shed the shell, and the young began the life of a
so-called naked mollusk. The presence of a shell with
operculum in embryonic life of Nudibranchs has been ob-
served by various authors: Alder and Hancock, 1845;
Pelseneer, 1893; Smith, Bell and Kirkpatrick, 1905; Boas,
1916, and others.

A large number of eggs were present in each capsule of the first nidosome (Figs. 4–7), and all developed into
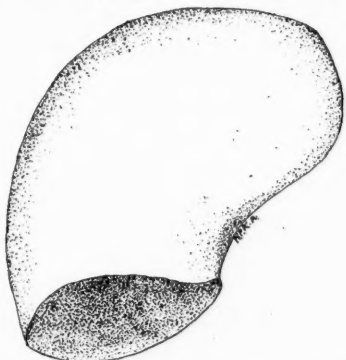

FIG. 10. Embryonic shell of *Melibe leonina.*

embryos which finally went through metamorphosis. In the second nidosome, some of the eggs failed to develop. The cause was perhaps lack of spermatozoa. During copulation, the spermatozoa become stored in the spermatotheca but also wander up the uterus as far as the prostate gland (Fig. 11, *spt., ovd., pr.*). The eggs are probably fertilized while passing down the uterus, *ut.,* or while in the spermatotheca, *spt.,* as many eggs actually pass into this out-pocketing of the uterus, the spermatotheca may therefore be termed ovo-spermatotheca. The only means of regulating the fertilization process in the egg must be the physical condition of the egg, which determines the reception or the function of the sperm; as all eggs, under natural conditions, contained in one and the same capsule, and, indeed, in the entire nidosome, go through simultaneous development, although they all (perhaps more than 100,000, in one normal deposit, nidosome) can not possibly have been fertilized at the same moment. This primary part of fertilization must take place before the eggs are incapsulated. If, however, an insufficient number of spermatozoa are present during the flow of the eggs, some eggs may become incapsulated without having been fertilized. One thing noted was

that the eggs in one end of the nidosomic belt, and in the main part of it, were all fertilized, while the other end (the last end) of the belt showed lack of fertilization. Another fact noted is that *Melibe leonina* carries over spermatozoa in its genital vessels; that more than one egg-body is deposited after insemination.
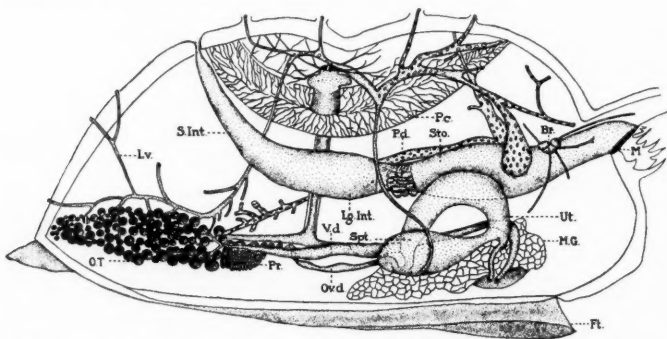

Fig. 11. Schematic drawing of dissected adult animal to show the general arrangement of the visceral organs : *Br*, brains; *Ft*, foot; *Lg. int*, anterior and large part of the intestine; *M*, mouth; *Mg*, mucous gland; *Ov. d*, oviduct; *Pc*, pericardium; *Pr*, prostate; *Si*, small part of the intestine; *Sto*, stomach; *Ut*, uterus; *Vd*, vas deferens; *Lv*, branch of liver; *Spt*, ovo-spermatotheca.

Reid's observations, 1846, on *Doris bilamellata*, *D. tuberculata*, *Gonidoris barvicensis*, *Polycera quadrilineata*, *Dendronotus arborescens*, *Doto coronata*, and a species of *Eolis*, bring out the same fact, viz., more than one deposit takes place after insemination. From 26 hours after coitus deposition may begin; '' it does not, however, appear to be absolutely necessary for the production of fertile ova in all, if in any of the individuals of the nudibranchiate Mollusca, that coitus should have so shortly preceded spawning as was observed in Polycera, for an Eolis which was kept strictly confined in a vessel by itself, deposited, on the tenth and again on the thirty-second day of its isolation, abundance of fertile ova.'' Crozier, 1919, claims that the larger animals of *Chromodoris zebra* Heilprin, lay several more egg-masses in a given time than do small ones; that it is consequently of advantage to the species that large individuals

should mate together; that there is, in fact, selective pairing which is of a distinctly advantageous or "purposeful" character, since it makes for the multiplication of the species. The same author, 1917*a*, records the unique findings relative to a rather high degree of correlation between the sizes of the two pairing members of *Chromodoris zebra*. The writer has observed the same fact relative to copulation among the Eolidæ. As for *Melibe*
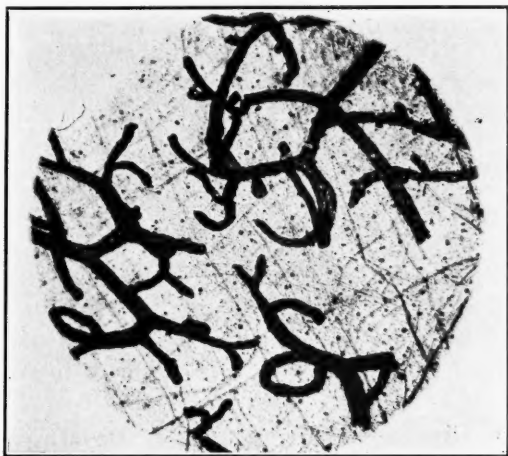

FIG. 12. Micro-photograph of the inner side of the ectoderm of the body-wall, showing the odoriferous glands, the small dots among the branching (black) hepatic cæca, and the crossing muscle (pale) fibers.

leonina, it is also true that those found copulating were of the same relative size.   But even so, as seen in the second nidosome of *Melibe,* the animal may run short of spermatozoa during ova-deposition.   To guard against this, there is, as shown by Crozier, among certain species, selective pairing between individuals of nearly the same size.   Garstang, 1890, finds a considerable variation between the offspring of *Lomanotus* Vérany, "the individuals apparently showing a tendency to unite rather with those of their own variety than with those unlike themselves."

To understand the process of insemination of the eggs, and the conditions controlling the number of eggs in the capsule, a little speculation is necessary. It seems as if the processes of fertilization and incapsulation are effected during the emission of the eggs; that when the eggs pass down the uterus or pass the spermatotheca they are fertilized, and immediately after that incapsulated, and that the size of the capsules and the number of eggs present in each capsule are regulated by the speed of the outflow of the eggs. The size of the capsule, as a rule, varies according to the number of the eggs present within the capsule. It seems puzzling, however, when capsules are found without eggs, and with eggs which show no indication of being fertilized, but this abnormality is limited to the last part of the nidosomic belt, and is of course so controlled that an entire egg-body may not be deposited without some eggs at least being present, and being fertilized. It is a matter for future observation to determine whether individuals in ovamaturity are capable of depositing normal nidosomes, without being stimulated by an individual in ripe malephase. It is a question whether the mere pressure of ripening eggs will cause egg-flow. Crozier, 1917*b*, reports, however, that *Chromodoris zebra,* if left alone, deposits fragments of egg-bodies which are not fertilized. The writer has noted the same phenomenon relative to *Eolis olivacea* when it is kept alone; but also in this case, as in the case of *Chromodoris,* no normal nidosomes were deposited.

The question of cross-fertilization becomes of interest since spermatozoa are found in both genital ducts of the same individual, from the ampulla of the penis and all the way down the penis to the end of it; from and including the prostate which surrounds the uterus, to and including the ovo-spermatotheca. If self-fertilization takes place, should there be any shortage of spermatozoa during ovadeposition? The presence of spermatozoa in the female genital tube is undoubtedly the result of coition and the

wandering of the spermatozoa up the uterus, against the outward current of that organ.   Alder and Hancock, 1845, p. 25, say:

The Nudibranchs, notwithstanding that they are androgynous, frequently copulate during the breeding season.   The conjoined individuals lie side by side, their heads turned in opposite directions.   Thus the right sides of the two animals are brought in close contact, and mutual impregnation is effected.   They remain in this position for some time, but in a short period after separating, generally about the first or second day, the spawn is deposited.

Crozier, 1919, claims that *Chromodoris zebra* is functionally hermaphroditic, and effective reciprocal insemination is practised.   But this is not practised among the species *Melibe leonina;* although semen may be present at the same time in both genital ducts, insemination is not reciprocated simultaneously.   That is, in all the individuals examined, coitus was effected by the introduction of the penis of the one mate; the penis of the other mate was completely withdrawn.   Whether spermatozoa were present in the members whose external genital organs were not visible was not determined.   It looks, however, as if *Melibe leonina* is protandric, a condition, according to Pelseneer, 1895, common among *Eolis, Elysia,* and *Clione limocina.*   Eliot, 1910, says:

Pairing, according to Hecht, is reciprocal, and though hermaphrodite Mollusca are incapable of self-impregnation both individuals spawn after mating.

The writer has observed on *Eolis olivacea,* at Woods Hole, that one mate may start spawning while copulating. Spermatozoa, according to Reid, may be carried in the female genitals (*Eolis*) for more than thirty days before being used.   That is, cleavage does not start in the eggs of *Eolis* until after deposition; fertilization, therefore, may not occur before the time of incapsulation.   Spermatozoa are kept alive in, and stimulated by, secretions of the female genital organs, as shown by Eliot and Evans, 1908, p. 287:

The walls of the spermatotheca (of *Doridoides gardineri*) are thick
and produce a secretion. In some specimens small clumps of sper-
matoza are embedded in this secretion. In others all the spermatozoa
form a central mass in the main cavity of the spermatotheca. It is
possible that the secretion serves to form small packets of spermatozoa
or spermatophores.

Summary

1. *Melibe leonina* is a large carnivorous Nudibranch
reaching sometimes 14 centimeters in total length; it is
an actively predaceous animal; it practically gorges
itself, feeding mainly on small Crustacea; it is gregarious.

2. It seems to live more than one year; its recurrence
is spasmodic.

3. It swims freely in the water, backward, upward or
downward; it crawls on the surface by the surface ten-
sion, and on sea-weeds, by the help of its highly ciliated
foot.

4. Its defensive means are an offensive odor and death
feigning.

5. It drops to deeper water by relaxation of its
muscles.

6. It collects in groups among sea-weeds, where copu-
lation takes place.

7. Mutual insemination does not seem to be simul-
taneous.

8. It spawns as early as March and as late as July;
sexual maturity is reached quite early, as young ones
two centimeters long were found with ripe spermatozoa.

9. Spermatozoa from another individual are stored in
the ovo-spermatotheca but wander up the uterus as far
as the prostate.

10. Eggs are also stored in the spermatotheca, hence
the name ovo-spermatotheca.

11. Copulating individuals are of the same relative size.

12. The same individual deposits more than one nido-
some, after insemination; spermatozoa may be carried
over in the ovo-spermatotheca at least two weeks.

13. The eggs are deposited in capsules, normally containing from 15 to 22 eggs.   The capsules are arranged in rows within a gelatinous mass, sometimes quite regularly; the gelatinous mass is formed into a belt from 3 to 5 cm. wide; the mucous flow is greater in one side of the belt than in the other, so that one side of the belt is shorter than the other, and the belt curves into a funnel-shaped mass, the apex adhering to some sea-weed, near the surface of the water.

14. Eggs may become incapsulated without being fertilized; no cleavage of such. eggs follows.

15. Normally the embryo develops within two weeks.

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TYPES OF MUTATIONS AND THEIR POSSIBLE SIGNIFICANCE IN EVOLUTION.[1]

DR. ALBERT F. BLAKESLEE

STATION FOR EXPERIMENTAL EVOLUTION

THE beginning of the twentieth century saw the rise of two concepts which have profoundly affected biological thought and been of increasing influence in the trend of experimental study of plants and animals. The mutation theory of deVries based on the evening primrose, and the laws of Mendel based on the garden pea, settled the date of birth of the modern science of genetics. The studies on these two plants have together formed a basis for the main bulk of our present genetic investigations. While the garden pea stands intimately associated with a conception of inheritance of wider application than was at first imagined, the evening primrose and the theory of mutation connected with it are by many considered to furnish an example of a valuable theory founded upon incorrect interpretations. The belief is growing that most of the new forms which have appeared in cultures of the Œnotheras are not mutations at all and that the evening primroses, as an abnormal group of plants, are not to be seriously considered as representative of the processes of evolution in normal forms.

In the short time at my disposal, I wish to outline some recent findings in the jimson weed (*Datura Stramonium*) which it is hoped may throw incidentally some light on the more highly involved phenomena in the Œnotheras, and which may serve as a basis of a brief discussion of their possible evolutionary significance.

The jimson weed is not supplied with a wide range of obvious Mendelian characters. The early studies of

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[1] A paper presented before the American Society of Naturalists at the Chicago meeting, December 30, 1920.

Naudin (9) and Godron (7) as well as the later investigations of deVries (13), Bateson and Saunders (1) and the writer and Avery (4) on this species have shown that purple color in flower and stem is dominant to lack of purple in those parts, and that spiny capsules are dominant to smooth capsules. The writer with Avery (5) has been able to add a third pair of contrasting characters: "many nodes," causing tall stature, in contrast to "few nodes," causing low stature. These are all the allelomorphic pairs actually determined. Moreover, no variation has arisen in the writer's cultures during the last seven years' study of this species which gave evidence of differing from the present stock by a single Mendelian factor. Distinct variations, provisionally termed mutations, have, however, regularly recurred whenever a sufficiently large number of plants have been subjected to observation (5). So far as investigated, they have been found to be connected with a duplication of one or more of the normal chromosomes (6). The normal quota is 12 pairs: 12 being therefore the gametic, haploid, or x number; and 24 the somatic, diploid, or 2x number. The simplest type of duplication is the addition of an extra chromosome, probably by non-disjunction in one of the pairs of the diploid complement, giving $24 + 1$, or 25 chromosomes as the somatic number. In such plants there will be 11 sets of 2 homologous chromosomes each and 1 set of 3 homologous chromosomes.

We have on the chart, Table I, 12 recurrent mutants of the type just discussed; which, while perfectly distinct from each other and from the normal stock, have certain characteristics in common. All have been found to produce gametes with 12 *and* 13 chromosomes (therefore with 25 as the calculated somatic number); all have a relatively large proportion of bad pollen grains, varying in the different mutants from 8 per cent. in the Globe to 21 per cent. in the Spinach, as indicated in the chart; all fail to transmit the mutant complex to any considerable extent through the pollen, while they do

TABLE I

SOMATIC NUMBER OF CHROMOSOMES AND PERCENTAGE OF BAD POLLEN
FOUND IN NORMALS AND IN DIFFERENT MUTANTS

Types	Somatic Number of Chromosomes	Per Cent. Bad Pollen
NORMALS	24	2.7
MUTANTS		
Tetraploid ("New Species")	48	3.3
Triploid	36	34.1
Simple Trisomic		
1. Globe	25	7.9
2. Poinsettia	25	12.9
2a. P. var. wiry	25	9.3
3. Cocklebur	25	18.3
4. Ilex	25	12.2
5. Mutilated	25	20.7
6. Sugar loaf	25	16.1
7. Rolled	25	8.4
8. Reduced	25	10.7
9. Buckling	25	10.4
10. Glossy	25	18.0
11. Microcarpic	25	12.8
12. Spinach	25	20.7

transmit it through the egg cells, although to only about one quarter of the offspring. That the offspring of these mutants repeat the parental type regularly in less than the 50 per cent. expected is probably due to the lessened vigor of growth of mutants in comparison with normals.

If the presence of an extra chromosome in a given set causes a specific mutation due to the constitution of this particular chromosome, rather than to the mere presence of an extra chromosome irrespective of its origin, there are at least two consequences to be expected. First there should be as many possible mutants of this type as there are chromosome sets which may undergo duplication. In other words there should be 12. Twelve, as a matter of fact, is the actual number which we had found before the nuclear condition had been determined. In addition, we have two or three mutant forms apparently belonging to this class for which it has not yet been possible to obtain chromosome counts. In appearance they are combinations or modifications of members of the

recurrent twelve. Secondly, it should be possible by breeding tests to connect up mutants with as many chromosome sets as there are known Mendelian factors, or factor groups. This connection we seem to have established between the mutant Poinsettia and the set of chromosomes which carries the factors for purple pigmentation in flower and stem.

TABLE II

TYPES OF CHROMOSOMAL DUPLICATION, GAMETIC AND SOMATIC FORMULAE
FOR PLANTS HETEROZYGOUS FOR FACTOR PAIR A AND a AND RATIOS
OBTAINED WHEN SUCH PLANTS ARE SELFED, TOGETHER
WITH DIAGRAMS ILLUSTRATING THE CHROMO-
SOMAL CONDITION IN SOMATIC CELLS

No. of Extra Chromosomes in Set	No. of Sets Affected	Gametic Formula	Selfed Ratios	Somatic Formula	Somatic Diagram
2	12	AA + Aa AA + 4Aa + aa Aa + aa (12 + 12)	1A : 0a 35A : 1a 3A : 1a	AAAa AAaa Aaaa (12 + 12) + (12 + 12)	
1	1	2A + a + AA + 2Aa A + 2a + 2Aa + aa 12, (12 + 1)	NOR. 8A : 1a MUT. 9A : 0a NOR. 5A : 4a MUT. 7A : 2a	} AAa } Aaa (12 + 12) + 1	
1	12			(12 + 12) + 12	

No. of chromosomes 12 +	0	1	2	3	4	5	6	7	8	9	10	11	12
Frequencies	1	12	66	220	495	792	924	792	495	220	66	12	1

The set of 3 chromosomes in the diagram, Table II, may be called the Poinsettia set, or the purple set. A Poinsettia plant may, to speak in terms of the dominant factor, be considered nulliplex with no dominant genes, or simplex, duplex or triplex with, respectively, 1, 2, or 3 dominant factors. There are therefore two types of heterozygotes, and under greenhouse conditions these apparently can be distinguished from each other as well as

from the homozygous dominants by different intensities of pigmentation. Simplex heterozygotes when selfed throw offspring with 5 dominants to 4 recessives among the normals, and 7 dominants to 2 recessives among the Poinsettias; while duplex heterozygotes should give a ratio of 8:1 among the normals, and all dominants among the Poinsettias. There is evidence which seems to indicate that the mutant Cocklebur is conditioned by duplication in the chromosome set which carries both the factors for spiny capsules and also those for number of nodes. If this is actually the case, we must assume that these two factor pairs are loosely linked in the same chromosome with about 50 per cent. crossing over, since they appear to segregate independently of each other.

We have been discussing duplication of a single member in only one of the 12 chromosome sets. On the lower part of the chart (Table II) is represented the only plant we have yet found with an extra chromosome in every one of its 12 sets. Such a plant is triploid. What its breeding behavior will be, can not be told before another season. If the chromosomes assort at random, the gametes theoretically should have the chromosome numbers indicated in the chart, and the counts which my colleague, Mr. Belling, has made from figures in pollen mother cells are not inconsistent with the distribution of the theoretical frequencies. One might expect such triploid plants to give rise to individuals intermediate between triploids and mutants of the Poinsettia type; in other words to mutants with duplication of chromosomes in 2, 3, 4, etc., up to duplication in all the 12 sets. Such compound mutants we have not yet been able to surely identify in our cultures; but we have never before this past season had a triploid plant, which from the wide range of gametic types in its egg cells would seem a likely source of such mutations.

Tetraploid plants have been discussed at yesterday's session of the Botanical Society of America. They represent a further duplication over those of the triploids

already mentioned in that there are 4 homologous chromosomes in each set in somatic cells. The homologous chromosomes therefore form tetrasomes, to use a new term,[2] instead of disomes as in normals or trisomes as in triploid plants. Members of these tetrasomes apparently assort at random in the reduction division. In consequence, certain peculiarities in breeding behavior result. Plants duplex for a dominant factor (AAaa) will, when selfed, give a ratio of 35 dominants to 1 recessive in the offspring. Plants simplex for the dominant (Aaaa) will give a 3:1 ratio in their offspring; but a third of the dominant offspring will throw 35:1 ratios in the next generation. Plants triplex for the dominant (AAAa) will give in the immediate offspring all dominants; one quarter of which, however, may be expected to give a 35:1 ratio in a later generation. The results expected from selfing the 5 zygotic types are shown in Table III.

It might be expected that mutant forms would be found in which doubling of the chromosomal number had involved only a single one of the 12 sets. Such mutants would bear the same relation to tetraploid plants with all the sets involved that the Poinsettia type of mutants bear to triploid plants. They have not yet been found, however.

[2] The following terms are suggested to designate sets with numbers of chromosomes from 1 to 12: monosome, disome, trisome, tetrasome, pentasome, hexasome, heptasome, oktasome, enneasome, dekasome, hendekasome, dodekasome.

The number of sets affected by duplication may be indicated by the terms: simple, double, triple, quadruple, quintuple, sextuple, septuple, octuple, nonuple, decuple, undecuple, duodecuple.

The Poinsettia and Globe are simple trisomic mutants. If the Globe and Poinsettia could be combined to form a mutant with 3 chromosomes each in two of the 12 sets, such a mutant would be called a double trisomic mutant. If differential viability of gametes does not interfere, the triploid plant already mentioned should produce, theoretically, offspring of all the trisomic types from simple to duodecuple. Haploid, diploid, triploid, tetraploid, etc., are terms already employed to designate plants with the same number of chromosomes in all the sets.

TABLE III

TETRAPLOID PLANTS. RESULTS OF SELFING TETRAPLOID PLANTS ARISING FROM THE CROSS OF A HOMOZYGOUS DOMINANT (AAAA) BY A RECESSIVE (aaaa), CARRIED TO THE F_4 GENERATION

In the F_4, only phenotypes are represented.

P_1— AAAA and aaaa

F_1—AAaa   (Gametes of F_1 — AA + 4Aa + aa)

F_2—1AAAA   +   8AAAa   +   18AAaa   +   8Aaaa   +   1aaaa

F_3—AAAA   AAAA + 2AAAa + AAaa   AAaa + 2Aaaa + aaaa   aaaa

F_4—A   A   A   35A: 1a   35A: 1a   3A: 1a   a   a

It is possible that a single set in an otherwise tetraploid plant may have an extra chromosome, giving 5 chromosomes in one set and 4 in the remaining eleven. At least we have a single plant in a tetraploid pedigree which strongly resembles the Globe—the best known of our simple trisomic mutants. The cytological evidence shows that its chromosomal number is at least tetraploid, but is not yet sufficient to prove that its Globe-like appearance is determined by the addition of a fifth member to the chromosomal set responsible for the Globe mutant.

The occurrence of mutations of the types discussed in the foregoing paragraphs is bound up with the causes of chromosomal duplication. Knowing the mechanism to be affected, we may be able ultimately to induce chromosomal mutations by the application of appropriate stimuli.

We have outlined the types of chromosomal duplication already found in the jimson weed, and have shown some of the peculiarities in the breeding behavior of the mutant forms which they condition. It will be well to consider for a moment this process of duplication as it affects the individual plant and as it may have a possible significance in our theories of mutation and evolution.

The mutants of the Poinsettia or Globe type, in which but a single chromosomal set is involved in the duplication, should enable one to discover something in regard

to the influence of each specific chromosome upon the morphology and physiology of the datura plant. While there seems to be but a single chromosomal set responsible for the presence or absence of purple pigmentation, probably each chromosome has an influence upon the strength of expression of the pigment since the several mutants appear to differ widely in color when homozygous for the main purple factor. Thus Glossy is darker purple than normals, while Cocklebur is distinctly lighter. In normal plants there is a balanced adjustment between the modifying factors in the different chromosomes. When this balance is disturbed by the addition of only a single extra chromosome to one of the 12 sets, profound changes are brought about in the ontogeny of the resultant plant. When all of the sets have an extra chromosome, however, as is the case in triploids, no great disturbance of the balance is brought about and the plant is not greatly different from normals. Even in tetraploid plants where all the sets are equally affected, although the total number of chromosomes is doubled, the difference from normal is not so great as in mutants of the Globe and Poinsettia series. The leaves of tetraploid plants, when carrying the factor for many nodes, may be distinctly larger than those of normals. Few-noded tetraploids, however, are less easily distinguished. The best diagnostic character has been the globose shape of the capsule, and yet plants known to be tetraploid from cytological evidence have been found this past season with capsules perfectly normal in appearance.

What is the bearing of the phenomena of chromosomal duplication in datura upon the mutation theory? In the first place, the mutants of the Globe type apparently correspond to the *lata* type of mutants in the Œnotheras in which an odd somatic chromosome has been determined, although in these Œnothera mutants no breeding evidence has been available to show that the peculiarities of mutant *lata* are due to the presence of an extra chromosome in any specific chromosomal set. Our tetraploid

mutant "New Species" corresponds to *Œnothera gigas*
and is brought about by a doubling of the chromosome
number. The color ratios in our tetraploid daturas indi-
cate that *Œnothera nanella* is a Mendelian segregate and
suggest that other of the Œnothera mutants which give
monohybrid ratios in crosses may be of the same nature.
Our evidence in regard to *O. nanella* comes from the oc-
currence of this mutant in cultures of *O. gigas*. DeVries
(14) reports that certain races of *gigas* when selfed regu-
larly produce from 1 to 2 per cent. *nanella* mutants, while
certain pedigrees give monohybrid ratios which, on ac-
count of the lesser vitality of the recessive *nanellas,* show
a higher proportion of the dominant *gigas* forms. From
the pedigrees approaching a 3:1 ratio he obtained plants
which bred true, except again for the 1 to 2 per cent. of
nanella mutants in their offspring. A glance at the chart
(Table III) will show that, if our theory of tetraploidy
be correct, the 1 to 2 per cent. of mutant *nanellas* which
deVries obtained by selfing plants from 3:1 pedigrees
must have been the recessives in a 35:1 ratio since no
dominant plants in a 3:1 pedigree of a tetraploid race
could be expected to breed true. The dominant pheno-
types must either throw 3:1 ratios again or 35:1 ratios.
The deviations of the *nanella* mutants in this case from a
35:1 ratio is accounted for by a similar proportionate
deviation in the 3:1 ratio. The work of Muller (8) on
balanced lethals strongly suggests that such of the Œno-
thera mutants as are not caused by chromosomal duplica-
tion are due to cross-overs from a balanced lethal con-
dition.

What then is a mutation? I do not feel we need to be
bound by its application to the evening primrose for
reasons of priority, since Waagen (15) had previously
used the term in paleontology in an entirely different
sense. I believe, with the idea that mutations must in-
volve a qualitative change, that we shall ultimately con-
fine the term to mutations of genes, although such muta-
tions may later be shown to be as different from our

present conceptions of them as are mutations in the Œnotheras from the conceptions in deVries's classical publication, "The Mutation Theory." It may still be desirable to employ the word *mutation* as a collective term to designate the sudden appearance of any apparent genetic novelty—whatever its real cause—until we know better. Strictly speaking I should not call chromosomal aberrations mutations when the changes are purely quantitative. The occurrence of tetraploidy would therefore be no more a mutation than the doubling of chromosomes at the origin of the sporophyte from the gametophyte of ferns.

We have seen that chromosomal duplications and related phenomena may simulate gene mutations in their effects upon the individual. What is their possible significance in evolution? Let us first consider tetraploidy. Numerous investigators have called attention to the fact that the chromosome numbers of plants are more frequently in multiples of two and four than one would expect from random sampling. Pairs of related species have been listed for which one member had twice as many chromosomes as the other. Such species have even been called tetraploid. We feel strongly the desirability of confining the term tetraploidy to those cases in which the 4x number is brought about by a doubling of homologous chromosomes. Doubling by transverse division is a possible method, but would not be included in the term.

Tetraploidy has been observed in experimental cultures of œnothera, primula and datura. Do such tetraploid plants occur in nature, and are they capable of giving rise to taxonomically new species? It may be mentioned that the tetraploid datura was called " New Species " before its tetraploid nature was suspected. It satisfied the requirements of an independent species. The pollen was relatively good, and the mutant formed a distinct race, self-fertile and fertile *inter se,* while practically sterile with the parent stock. Tetraploid plants, therefore, stand slight chance of being swamped by hybridization with the

species from which they have sprung. Once arisen, their chances of survival would depend upon their ability to complete with other forms in the struggle for existence. There are no certain cases of tetraploidy known outside of cultivation. It must be admitted, however, that their identification would be difficult. I have shown that gigantism is not an invariable diagnostic feature of tetraploid daturas. As yet no cytological criteria of tetraploidy have been established. The breeding behavior, which is the only safe test, might easily be misinterpreted, as it was apparently by deVries in the case of the tetraploid *gigas* and *nanella*. Moreover, a suspected form must show a pair of Mendelizing characters before a breeding test can be applied.

Despite the paucity of evidence in regard to the occurrence of tetraploidy in nature, the speaker believes that it may have been one of the principal methods in the evolution of plants. Its occurrence would furnish the barrier between a new species and its parental form that Darwin sought, and it would give a reason for the prevalence of even numbers in the counts of chromosome pairs. I believe that a search for tetraploid forms in nature will be rewarded. Perhaps they will more likely be found in horticultural races propagated by vegetative means. I take this occasion to suggest the desirability of testing for tetraploidy any *gigas*-like plant that may be found in the wild or under cultivation. We are making a special study of tetraploidy at the Station for Experimental Evolution and should be glad to receive plants suspected of being tetraploid from any who do not care to make the necessary tests themselves.

Even if proper tests should show that few forms in nature were tetraploid in the sense that each chromosomal set in somatic cells was composed of 4 homologous members, tetraploidy might still be a stage in the origin of species with an even number of pairs of chromosomes. In the 3 forms in which tetraploid plants have arisen under observation, the 4 homologous chromosomes in a

set apparently assort at random in the reduction division. If, instead of acting individually without predilection one for another, the four should come to assort in pairs, we should have a different ratio in the F_2 generation ($15:1$ instead of $35:1$). There would still be duplication of genes and a 4x number in reference to the parental form, but independent assortment of the chromosomes would have been lost. It will not be possible to go into the details of the argument. It is at least suggestive that Shull (12) has found 3 cases of duplicate genes in the shepherd's purse, which has 32 chromosomes (that is 4 times 8); and Nilson-Ehle (10) has found a case of triplicate genes in a wheat having 42 chromosomes, which is 3 times the number in another variety of wheat (11).

If tetraploid plants have been of influence in evolution, it is probable that the other types of duplication have also been of influence. A mutant of the Globe type with a single duplication in one of the 12 sets ordinarily fails to hand on the duplication through the pollen. Occasionally it might do so, and we should then expect a constant race with 4 homologous chromosomes in one of the 12 sets. If these 4 should cease to assort at random and pair, we should again have a possible duplication of genes and an added pair of chromosomes characteristic of the race.

There is not time at my disposal to discuss mutations of genes. In a recent paper (2) on a somatic mutation in portulaca, I have indicated my belief that mutations of genes may occur at any stage in the development of the plant. We have found color mutations which affected only the epidermis, and therefore could not be transmitted through seeds. We have also found similar color mutations which affected only the sub-epidermal tissue, and therefore could not show in the petal; but which became evident from the seeds produced from this mutated tissue. There seems to be no preferred location for the origin of factorial mutations in flowering plants, although they are more readily transmitted if they occur in the

gametes or in the embryo. The fact that in vegetatively propagated Mucors (3) I have found mutations relatively common where the possibility of sexual reproduction was ruled out, indicates that sudden genetic changes are not necessarily associated with sexual processes.

It has not been possible in this brief presentation to give an extended classification of mutations, nor to discuss in detail their possible significance in evolution. It will be sufficient if I have made clear the distinction which must be kept in mind, in any discussion of the subject, between mutations in individual genes and those brought about by chromosomal aberrations.

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BOOKS AND LITERATURE

Die Chromosomenzahl von Zea Mays L. Ein Beitrag zur Hypothese der Individualität der Chromosomen und zur Frage über die Herkunft von Zea Mays L.[1] By YOSHINARI KUWADA.

The author of the paper, the title of which appears above, has well summed up his purpose in the subheading. As this article reports investigations of considerable cytological importance in a publication which is not likely to have wide circulation in America, it was thought advisable to review it at some length.

As Professor Kuwada clearly and concisely states his results and conclusions in his summary a translation is given below.

1. The chromosome number of *Zea Mays* L. is 10 (when the diploid number is 20). In forms either closely related systematically or supposedly ancestral types the chromosome number in the root tips is in general constantly 20 (seldom does the number approach the octoploid number).

2. It has been found that in one race of sugar corn which I received from the Agricultural Faculty of the Imperial University of Tokyo the chromosome number is different in different individuals. In the roots tips 21, 21, 22, 23 and 24 chromosomes were found. The number of tetrads is correspondingly different, namely, 10, 11 and 12. There is no relation between the chromosome number and the chemical nature of the endosperm.

3. Through a parallel study of the number and size of the tetrads and the length of the chromosomes in the root tips it has been shown that the number of chromosomes is increased through the cross fragmentation of certain chromosomes.

4. The measurement of the chromosome length in the root tips and the unequal lengths of the component elements of the tetrads allow us to draw the important conclusion that *Zea Mays* is of hybrid nature, and indeed, as Collins has rightly said, a hybrid between Euchlæna and an unknown plant of the Andropogoneæ.

5. The chromosomes supposedly derived from *Euchlæna* are longer than those coming from the Andropogoneæ species, so that the tetrads under certain circumstances are made up of elements of different lengths. The two chromosomes of the first kind A—B[2] and C—D have

[1] *Jour. of the Col. of Science*, Imp. Univ. of Tokyo, Vol. 39, Art. 10, 1919.
[2] It has been necessary for convenience to take some liberties with the method used by Kuwada for expressing his idea of the potentiality of fragmentation possessed by the various chromosomes. Here a solid dash be-

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an inclination to fragment easily under certain conditions while the corresponding chromosomes of the latter type a b and c d do not show this tendency. In one plant (sugar corn) from the agricultural faculty of the University of Tokyo the chromosomes A—B and C—D have each cross fragmented into two chromosomes A B and C D and this condition is morphologically and genetically fixed. We therefore have three kinds of corresponding chromosomes: the cross-fragmented chromosomes, those having a tendency to fragment and those in which both of these characteristics are lacking.

6. In the formation of the tetrads the chromosomes A B and C D are dominant to A—B and C—D and recessive to a b and c d. The dominance in the first case is somewhat unstable, so that the number of tetrads is subjected to fluctuation within certain limits. The difference in the behavior of the corresponding chromosomes A—B, C—D and a b, c d to A B C D is also a point in favor of Collins' hypothesis.

7. If the chromosomes A B and A—B form a tetrad in the reduction division four combinations result: A B, A—B, A—B and A- -B. The corresponding ends of the chromosomes A- and -B fuse relatively easily to reform the chromosome A—B. The possibility of fusion depends absolutely on the proximity of the corresponding ends of the passive cross-fragmented chromosome A- and -B. In this respect the parallel arrangement of the homologous chromosomes in the somatic cells is of great importance. The chromosomes A and -B or A- and B which would otherwise occasionally fuse to form the chromosome A and -B or A- and B remain sometimes as A and -B or A- and B: the result being the variation in the number of the chromosomes. Two kinds of gametes occur, in one the chromosome number is constant and in the other it varies. The chromosomes in the first instance have the formula A B (number of chromosomes above normal) or A—B (normal number of chromosomes), and in the latter instance A—B or A- B or occasionally A- -B. When the chromosomes A B and a b form a tetrad the result is very simple in that only two combinations are possible—A B and a b. In these cases the number of chromosomes is constant. The union of A and B is only a phenomenon ascribed to the presence of a b.

The empirical results agree with those developed from theoretical considerations based on the laws of chance.

8. The applicability of the laws of chance to the chromosome num-

tween two letters indicates a weak place that may easily break, a broken dash before or after a letter suggests a free end of a fragment which will link up with the suitable end of another fragment if opportunity offers, underscored single letters have no power of uniting (no free ends), while the binding together of two letters by underscoring represents a chromosome which can never fragment.

ber and the constancy of the true length of the chromosomes in the
hybrids is a contribution favoring the individuality hypothesis.

9. The nuclear and cell size is dependent on the chromosome size
and on the other hand the latter is modified by the cell size.

According to Kuwada there are two hypotheses concerning the
origin of *Zea Mays* L. Iltis (1911) first suggested that this mod-
ern form might have been derived from some unknown tribe of
Andropogoneæ, while Collins in 1912 put forward the claim that
Zea Mays L. was a hybrid between an unknown species of the
Andropogoneæ and *Euchlæna*.

In his cytological studies Kuwada finds that in species of
Euchlæna and *Andropogoneæ* the chromosome number is the same
as in *Zea Mays* L.—namely 20. In only one of the investigated
groups of plants belonging to the *Andropogoneæ* was the chro-
mosome number above 20, which places this particular species
beyond consideration. The measurement of the chromosomes in
a *Euchlæna* from south Florida shows that their total length is
greater than is the case in *Andropogon Nardus* L. var. *Georingin*
Hack. The respective total chromosome lengths in each case are
given as 188.25 mm. and 111.3 mm.

Kuwada gives the results of a large number of measurements
of the chromosomes in various varieties of maize taken at random
or from plants in which the cytological conditions have been
studied in the parental, F_1 and F_2 generations. His conclusion
that the figures indicate that two length types of chromosomes
are concerned in the modern plant do not seem to the present
writer to be entirely born out by the facts.

In the measurement of chromosomes previous studies have
shown that complexes from the same individual in the same or
in different parts of the structure may show considerable varia-
tion in the total length of their component chromosomes. In
general, of course, small cells will have smaller chromosomes and
larger cells, larger chromosomes, but even in similar tissues very
appreciable differences may occur. These variations are ob-
viously due both to internal and to external causes. Fluctuation
in the climatic or nutritive conditions may affect growth and
vigor, while the volume of the cell imposes limitations on the size
of the contained chromosomes. It has been shown by the present
writer[3] that, be the total length of a complex long or short, the

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[3] Hance, R. T., 1917, ''The Diploid Chromosome Complexes of the Pig
(*Sus scrofa*) and their Variations, *Jour. Morph.*, Vol. 30. 1918a, '' Varia-
tions in the Number of Somatic Chromosomes in *Œnothera scintillans* De-

individual pairs always bear the same relation to each other, allowing the conclusion that whatever influences the size of the chromosomes generally affects all similarly. The figures of Kuwada bear out these observations very well. As pointed out above, his interpretation of his work seems somewhat forced. He recognizes the factors playing rôles in the behavior of the chromosomes, but does not feel that his results can be entirely explained by them.

To illustrate what is meant by the above criticism let us consider a cross made by Kuwada between sugar corn $22_{(15)}$ and Black Mexican $58_{(15)}$, another sugar corn. In both, dividing cells from adventitious roots were uniformly selected. The former has chromosome complexes averaging 149.05 mm. in length while the latter gives a total of 172.17 mm. This to Kuwada indicates a real and genetic difference in chromosome length, although in the same Black Mexican plant $58_{(15)}$ complexes from side root tips average only 145 mm. in length. This would signify that the length 172.11 was no more fundamental in plant $58_{(15)}$ than was 145, and lessens the weight of the evidence that the higher number betokens genetic chromosome differences with the length 149.05 in plant $22_{(15)}$. When the two plants are crossed the chromosome lengths in the hybrids are almost exactly one half of the sum of the lengths of these structures in the parents if 172.17 is accepted as the typical complex length for plant $58_{(15)}$— $1/2(149.05 + 172.17) = 160.61$. It may be pointed out here that one half the sum of the complex length found in the various roots of plant $58_{(15)}$ also closely approximates the same figure— $1/2(172.17 + 145) = 158.58$. The F_1 plants from the above cross possess sets of chromosomes whose length is very close to that expected on Kuwada's assumption of 149.05 and 172.17 as the basic or typical lengths of the parental chromosomes. The chromosomes in the F_1 plants varied from 155.75 mm. to 168.9 mm. and averaged 161.86 mm. This number fits in well with the anticipated result and at first would seem to justify the consideration of 172.17 mm. as the representative length for plant $58_{(15)}$. However, the chromosomes in the F_1 offspring were found in cells in the radicles of seed germinated in moist saw dust. The chromosomes in this early root tip in many forms are not infrequently larger than are found in the growing parts of

Vries, *Genetics*, Vol. 3. 1918*b*, ''Variations in Somatic Chromosomes,'' *Biol. Bull.*, Vol. 35.

the older plant and Kuwada's figures and statements show that maize is no exception to the general rule. This tendency for the chromosomes in the radicle to be larger puts a fictitious value on their measurements in this organ for comparison with the dimensions of chromosomes found elsewhere in the plant. As a matter of fact, in the number of examples given the average length of the chromosomes in all the plants is only a trifle more than one per cent. shorter than the similar data in regard to the chromosomes of the radicle, which difference would not greatly affect the end result. In this instance, although the physiological location of the chromosomes was undoubtedly one factor in determining their size, objection on this ground alone to the submission of the records of the F_1 chromosome lengths in substantiating the figure 172.17 as the fundamental chromosome length for plant $58_{(15)}$ would not seem to be entirely valid. However, to base a broad conclusion on the lengths of the chromosomes found in a particular part of a plant, even though comparing them with chromosomes from similar parts of other plants, is likely to obscure the real condition.

As has been shown in plant $58_{(15)}$, lengths of 172.17 mm. and 145 mm. were found. That these are not fixed lengths for the particular tissues concerned in this variety of corn is shown by the data given for other plants of the variety Black Mexican, in which lengths vary (for corresponding tissues) from 132.5 mm. to 181.25 mm., the average being 159.32 mm. There can be little question that the variety Black Mexican, as long as it is genetically pure, can have anything but comparable sets of chromosome throughout, holding in mind that though the lengths may vary the inter-pair relationship remains constant. Less variation in chromosome length is shown for the three plants of the variety "Sugar Corn" which were studied. The range of averages here is from 147.8 mm. to 151.6 mm.

Lastly, if real differences between the lengths of the chromosomes in plants $58_{(15)}$ and $22_{(15)}$ exist greater differences between the members of the pairs that are found in the hybrid offspring would be expected. Actually these elements mate up well as to length and if unequal homologous chromosomes have entered the zygotes union in a common environment has regulated their proportions. As the dimensions of the chromosomes are in part a function of their environment the selection as typical of any one complex or of even the average of com-

plexes from certain tissues only is not justified, considering our present ignorance of chromosome volume.

In support of the difference in length between the homologues of chromosome pairs as indicative of the genetic length types which Kuwada believes he has demonstrated he publishes drawings of tetrads showing the unequal length of the component elements. Personally I do not think that the figures are necessarily conclusive proof, since the arrangement of the homologues in several cases suggests a possible foreshortening, making the true length doubtful, and in other instances the drawings may well represent an entirely different form or stage of the tetrad. It is not the intention of this criticism to convey the impression that the investigator's figures fail absolutely in proving his point concerning the uneven length of the homologues, but rather to indicate that the illustrations are not nearly as satisfactory and as conclusive as those given in the publications of Wenrich[4]and Carothers[5] for somewhat similar conditions in other forms.

Between the *Euchlæna* and Andropogoneæ studied chromosome length differences appear which can scarcely be accounted for on the basis of environment. Since the characteristics of *Zea Mays* L. are intermediate between these forms the hope is raised that two sets of chromosomes will be found in the modern species, which hope the reviewer does not think has been realized. Indeed, though recognizing the evolutionary position of *Zea Mays* L. as given by some taxonomists, he offers the suggestion that in his opinion the present investigation has not, as far as the chromosomes are concerned, excluded the possibility of the origin by mutation of *Zea Mays* L. from *either Euchlæna* or the Andropogoneæ. A knowledge of the behavior of the chromosomes of these two forms in hybrids would be interesting and important.

In explanation of the variation in the number of chromosomes which Kuwada found in certain lines (20 to 24 chromosomes) he devised an exceedingly ingenious scheme which apparently thoroughly accounts for the numbers of chromosomes occurring in the offspring. It operates on the laws of chance and its theory

[4] Wenrich, D. H., 1916, ''The Spermatogenesis of *Phrynotettix magnus* and the Individuality of the Chromosomes, *Bull. Mus. Comp. Zool.*, Harvard College, Vol. 60.

[5] Carothers, E. E., 1913, ''The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes,'' *Jour. Morph.*, Vol. 24.

seems to be completely justified by the results. As this explanation is adequately outlined in the translated summary further space need not be devoted to it.

The investigator's theory of factors located in each chromomere which govern the form of the chromosome, while convenient in explaining the cause of the reunion of the chromosome fragments in maize, is scarcely necessary. Chromosomes are not inherited as are the determiners for adult characteristics in the form of minute chemical forerunners, but are passed on complete in all respects. Consequently, factors to determine their form in the next generation are not needed—the chromosome itself is carried over. The actual form of the chromosome has been shown by McClung, Wenrich, Carothers and others to be determined largely by the location of the spindle fiber attachment.

It is considered that the reviewed report has not clearly demonstrated the origin of *Zea Mays* L. by means of chromosome measurements for the following reasons:

1. The length of the selected chromosome complexes in the forms particularly studied are not typical of the plant and such selection gives a false impression of the actual conditions.

2. The figures illustrating the length differences of the homologues composing the tetrads are not entirely convincing or satisfactory.

3. If two types of genetically fixed chromosome lengths exist in maize we would expect to find an expression of this difference when both types enter into the same individual. As far as the reviewer's interpretation of the tables of length is concerned, this difference does not exist in the F_1 plants.

Though there are reasons for not considering that Kuwada has proved his claims of the origin of *Zea Mays* L. he, nevertheless, is to be sincerely congratulated on an excellent cytological contribution involving great labor and care. To the reviewer the apparent failure of Professor Kuwada to demonstrate his main thesis dwindles in importance when the value of the "side issues" of the investigation are considered. His work on *Zea Mays* L. presents the following data:

1. The chromosome pairs of a complex may be arranged in a graduated length series and between each pair there is an approximately equal difference in length.

2. The genetic relation of the chromosomes is shown in parent and offspring.

3. When chromosomes fragment in *Zea Mays* L. it is the longer ones that are affected. These fragments may also fuse, causing variability in the total chromosome number.

4. Suggestive methods of studying chromosomes have been devised.

5. Fragmentation has been accounted for on the basis of genetic tendencies and the variable number of the chromosomes in the offspring of certain plants has been ingeniously explained with the aid of the device described in his summary.

The first four points (with the exception of the latter part of the third, which has not been observed) agree perfectly with the reviewer's earlier work on the Œnotheras and the pig. As to the fifth point, he has never found fragmentation in the germ line.

Difficulties of interpretation in metrical studies of chromosomes arise from a lack of standards, *i.e.*, knowledge of the limits of variation that chromosomes of a given form will show under many conditions and of the uncertainty introduced by the personal equation involved in drawing and measuring. With the hope of deriving such standards the present writer is at work on a plant and an animal possessing very few chromosomes. The usefulness of the information drawn from such studies has been elsewhere discussed.

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SHORTER ARTICLES AND DISCUSSION
"HOMING" BEHAVIOR IN CHITON [1]

1. A STATEMENT concluding a recent preliminary account of the "homing" habits of the pulmonate *Onchidium floridanum* (Arey and Crozier, 1918) reads as follows:

> To the extent that the homing habits of *Onchidium* may be proved to involve associative memory, this snail may be placed in a series comprising such types as *Chiton, Fissurella, Onchidium* and *Octopus*, all four of which, in a sense, exhibit homing behavior, but of increasing degrees of precision and complexity in the order of arrangement here given.

The observations warranting a contention of this sort, so far as it involves *Chiton*, were not fully available when our analysis of the sensory responses of *Chiton tuberculatus* (Arey and Crozier, 1919) was written, and I have therefore considered it appropriate, as an addition to that report, to indicate the nature of the facts leading us to ascribe to *Chiton* a certain degree of "homing" behavior.

2. It was noted by Heath (1899, p. 4), on the Californian coast, that the adult *Ischnochiton magdalenensis* is found during the day under boulders between tides, but that at night the mollusc comes out to feed on seaweeds growing upon the rocks, retiring to dark situations after sunrise. Species of *Mopalia*, and *Cryptochiton*, were found to "remain out on their feeding grounds only when the day is foggy or dark." Numerous other species are more or less photonegative (cf. also Crozier, 1919b), but some nevertheless continuously occupy situations brilliantly illuminated (cf. Heath, 1899, p. 4; Plate, 1901; Pelseneer, 1906, p. 50). It has been shown elsewhere (Crozier and Arey, 1918; Arey and Crozier, 1919) that young individuals of *Chiton tuberculatus* are photonegative to ordinary daylight, the older ones photopositive. This matter of photic irritability is intimately concerned with certain diurnal movements simulating "homing" behavior.

Heath (1904) was of the opinion that the bilateral larval eyes of some chitons, persisting as they do well into postlarval life, until the shell plates become opaque, might be functionally im-

portant in determining responses to light.   Earlier, he had noted
(1899, p. 4) that the ventral soft parts of *Ischnochiton*, especially
the proboscis, might be sensitive to light, and it has been stated
in a general way (Pelseneer, 1906, p. 50) that chitons in which
there are no obvious shell "eyes" seem, nevertheless, to be sensi-
tive to illumination.   Direct proof has, however, been given by
Arey and myself (1919) that the tegmental æsthetes of *Chiton
tuberculatus* are photosensitive, and that this form of irritability
is important in determining the habitat of an individual chiton.

   C. tuberculatus attains a mean age of about 8 years (Crozier,
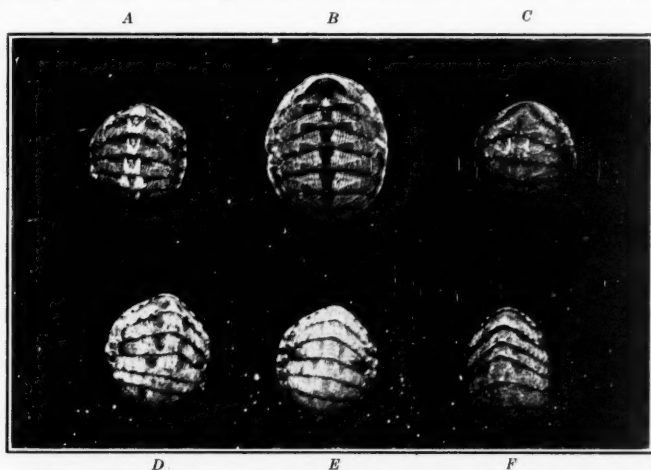1918*a*, 1918*b*).   As it grows, the periostracum and the surface of

FIG. 1.   Illustrating the relative lengths, ages and degrees of shell-erosion
in three groups of *Chiton tuberculatus* taken from localities quite near together,
but in the different situations indicated.   The extent of erosion seen in each
specimen was estimated by comparison with a graded series of "standard"
chitons: *A,* signifying *no erosion; B, slight erosion; C, a more severe stage, but
mild in comparison with D and E,* the last representing *relatively extreme
destruction of the tegmental surfaces.*

the tegmentum become eroded, and the superficial photoreceptive
organs destroyed (Arey and Crozier, 1919).   There is an almost
perfect correlation between the degree of this erosion and the
relative illumination of the situations frequented by these chitons.
The analysis of this state of affairs, and its implications, will be
more fully considered in another place, but as an illustration I
cite the following record, which is quite typical of many others:

   July 1, 1918.   At the northwest end of Marshall, Idaho, a small cove

bounded on the west side by rocks exposed to the sunlight of a cloud-
less day; the cove covered by loose slabs of rock, piled upon one
another; both situations, the exposed rock surface and the under sides
of the loose stones, yielded a number of chitons.

From the sunny rock surface, 38 individuals were obtained, ranging
in length from 5.5 to 8.9 cms., and in estimated age (Crozier, 1918a)
from 5 to 11 years. These were without exception seen to have the
tegmenta eroded to a greater or less extent.

From several crevices in the rock, having their deeper recesses well
sheltered from the sun, 8 specimens were secured. These were 4.5–6.9
cms. long, 4–6 years old, and but slightly eroded.

Under the stones, 30 chitons were collected. There were 3.4–6.9
cms. long, 2–7 years old, and at the most very mildly eroded.

Fig. 1 exhibits, for these lots, the distribution of: (1) length,
(2) age, and (3) degree of erosion. The "degree of erosion" of
the shell plates was judged empirically by comparison with a
graded series of "standard" examples, a method sufficiently pre-
cise for the purposes of this illustration.

It is obvious from the figure that those chitons with relatively
uneroded shells are younger, smaller, and live in darkened situa-
tions; whereas the older individuals, larger, with much eroded
shells, occur in the bright sunlight; those taken in partially il-
luminated cracks and crevices are of intermediate size and age,
and their shells exhibit an intermediate degree of erosion,[2] these
characteristics affording, in fact, an ethological definition of a
certain portion of the chiton population. The individuals of this
general "intermediate" class are frequently so situated that
they exhibit the type of "nocturnal" activity noted by Heath
(1899) for several species—they creep over open rock surfaces,
feeding, at night, and may remain out there on dark and gloomy
days, but return to crevices (or to the partial shelter of large
stones) when the rising sun is of ordinary brilliancy.

The uneroded chitons are photonegative to moonlight, even,
and although moving about actively at night, do not provide data
bearing on the possibility of "homing." This is largely true be-
cause the photonegative response of the younger, uneroded, chi-
tons becomes altered, depending upon the destruction of the teg-
mental receptors, in the directions of a photopositive reaction to
ordinary daylight; this alteration depends, not on age, but upon

[2] There are several methods of estimating rather precisely the exact amount
of erosion in any given case. In a later paper these methods are fully made
use of in analyzing the observed distributional occurrence of the Chitons.

the degree of intactiveness of the photoreceptive apparatus, and its importance in the present connection is due to the fact that the chitons of intermediate age, eroded to a moderate degree, and less photonegative[3] than the smaller ones, come to occur in places where moderate illumination prevails. Moreover, they move about much more freely than the younger individuals, thus often getting some distance away from loose rocks, not plentiful along many stretches of shore line. Crevices of one sort or another, or shaded depressions, are therefore the one type of refuge open to them.

3. It was found, by observing a group of marked chitons each day for a month, that the older ones and those of the "intermediate" group do not wander readily from place to place (Arey and Crozier, 1919). Another group containing 14 chitons of the "intermediate" class as above defined was under daily observation for three months, and the behavior of this group strongly suggested a feeble kind of "homing" phenomenon. The animals concerned spent most of the day under a boulder at half-tide. At night they crept out for distances of not more than a meter, feeding on *Enteromorpha* and other algæ. With the rising sun, they retreated to the rock-shelter. If the tide were out at sunrise, they remained more or less fixed until again covered by the sea, then moving toward the rock.

This sort of behavior, regularly and constantly exhibited, seemed to represent perhaps the incipient stages of a kind of "homing instinct."[4] The movements of the individuals of this group were therefore carefully watched. The bit of shore con-

[3] As worked out in a previous report (Arey and Crozier, 1919), the partial destruction of the photoreceptive apparatus through erosion is a principal factor conditioning lower stimulating power of light of a given intensity; in general, *Chiton* is actively photopositive to weak light, negative to intense light; with advancing age, therefore, the threshold for photonegativity becomes higher.

[4] Data regarding the movements of a single *Chiton* favorably situated on the side of a wharf and watched continuously for 9 months are given elsewhere (Arey and Crozier, 1919). This animal was quite old, and very much eroded. It remained in the open except during severe storms and one hurricane, when waves beat fiercely on the wharf. Under these circumstances the mollusc withdrew to a cavity at one end of the wharf, near the shore-line, and remained hidden. Several other cavities were available, but this particular one was automatically encountered as the *Chiton* moved shoreward on the wharf-side and away from the more exposed outer edge of the wharf. One wonders what the anectodotalist would make of a case like this!

cerned faced in general southeastward, and the tiny platform over which the chitons crept while feeding was so oriented with reference to the rising sun that the photonegative orientation of the animals and their subsequent creeping brought them for the most part automatically back to the shaded hollow under the rock. But I noticed repeatedly that in some instances the molluscs moved at an angle of 30°–40° across the direction of the sun's rays, moving more or less directly toward the rock. If such individuals were suddenly detached (with the aid of a cold-chisel and hammer, removing the animal still fixed to a bit of stone), and so placed as to necessitate its approaching the rock at a different angle, it usually did so without trouble. If re-moved to a greater distance than 1.5 meters, no return was ef-fected, the creature taking up a more or less permanent site in another shaded hollow.

Aside from light, it must be remembered that there are other possible directive agencies in such a case. The sea was rarely still, and even a slight tidal current would be sufficient to reflect pressure waves from the shore,—so that, perhaps by this means in part, a chiton would be oriented toward shore, and thus, in the present case, inevitably toward its rock-pocket. Additional specimens of the general "intermediate" group, brought from distant islands, were "planted" in this community, and engaged in the same nocturnal wanderings and early morning returns.

4. Without further analysis, the activities of a group of Chi-tons such as that described, may seem to involve a sort of be-havior resembling the well-known "homing" of *Fissurella*[5] and its allies. Yet with *Chiton* the matter is clearly less definite than in other instances recognized among molluscs, and, so far as I have seen, the facts may readily be interpreted in terms of im-mediate directive stimulation. There is nothing necessarily *specific* about the Chiton's "home." For this very reason such homing movements as *Chiton* may exhibit at a certain period of

[5] I encountered a curious instance of the "local habitation" affected by limpets, when examining the chitons of a reef on the south shore of Ber-muda. One of these chitons bore on its back a small *Fissurella*, the margin of the shell of which had become so modified that it fitted nicely one par-ticular spot on the much curved surface of the third valve of the chiton. Under water, the *Fissurella* wandered over the eroded shell of the large chiton, feeding upon epiphytic growths, but always returned to its "home." The *Chiton* was 9.3 cms., the *Fissurella* 0.9 cm. long.

its life-history may be taken to represent one extreme in the development of such behavior among molluscs, seen perhaps in its highest condition in *Octopus* (cf., *e.g.*, Cowdry, 1911).

<div align="right">W. J. Crozier</div>

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AN F_1 SPECIES CROSS BETWEEN HORDEUM VULGARE AND HORDEUM MURANIUM

WITHIN the last few years the subject of species hybridization has increasingly occupied the attention of those interested in the subject of heredity. The possibility of the genetic analysis of species hybrids depends upon the ability to cross and to secure offspring from the species in question. During the course of a plant-breeding investigation which was commenced at the

University of California, an attempt was made to determine if the common cultivated barley could be crossed with wild species of *Hordeum*. The wild species which were used were *H. nodosum* and *H. muranium*. For the sake of convenience the system of nomenclature proposed by H. V. Harlan (1918) will be adopted for the common varieties of barley used in the investigation.

One of the crosses which was attempted was between *H. vulgare vulgare pallidum* and *H. nodosum*. This cross was an entire failure, however, as no seeds were obtained from any of the flowers which were crossed. The anthers of the male parent were fully mature, and the plant which was used for the female parent was perfectly healthy and normal when the cross was attempted. As a matter of fact a successful cross was made the same day between a different head of the same plant of *H. vulgare vulgare pallidum* and *H. vulgare distichon palmella*. At the present time it would be difficult to say whether the absolute failure of this particular cross was due to the incompatibility of the gametes of the two parents or to certain errors of technique.

The other cross which was attempted was between *H. vulgare trifurcatum typica* and *H. muranium*. The contrast between the two parents was very marked and distinct. The low and often recumbent habit of growth of *muranium* was contrasted with the relatively tall and erect habit of *vulgare*. The light green leaves and stems of *muranium* were not nearly as stout as the gray green leaves and stems of *vulgare*. The spikes of the two species are also quite distinct. The spikes of *muranium* are compressed and composed of a number of rather narrow elongated spikelets which form rather a loose head. The spikes of *vulgare*, on the other hand, are generally composed of a number of relatively short and wide spikelets. Both species are annuals, but without going into further detail it is evident that there are a large number of morphological differences between these two species.

From the second cross two viable hybrid seeds were obtained. These grains resembled the typical seeds of the maternal variety in every respect. When they were planted, however, it required one and three days longer for the seed to germinate than for self-fertilized seed of the female parent.

The F_1 seedlings differed markedly from plants of *H. vulgare* in the same stage of development. The sheath or coleoptile had a greater circumference than the blade, thus fitting loosely

around it instead of adhering closely to the blade as in *vulgare*.
The sheath was closed along the side and open only at the apex.
The blade of the first leaf was narrow, linear and spirally
twisted with slightly roughened edges. The blade was about
one twelfth of an inch in width and tapered slightly toward the
apex (Fig. 1).

One plant grew to a height of four inches and developed roots
two to three inches long (see Fig. 2). The other plant devel-
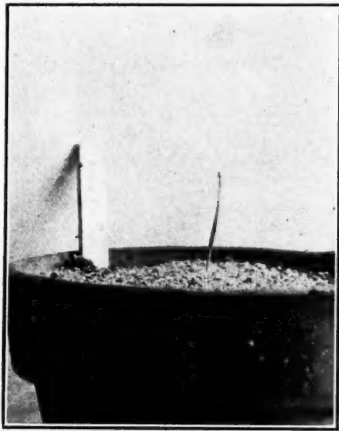oped somewhat more slowly, reaching a height of two inches

FIG. 1.   A first generation hybrid between *Hordeum vulgare trifurcatum typica*
and *Hordeum muranium*.

with roots of the same length. At this stage the plants ceased
development and gradually started to wither. Only one blade
was present and this extended to the seed. There was no evi-
dence of any nodes being formed.

Due to a change of residence the writer has not been able to
continue the investigation for a short time, but it is hoped that
this cross may be subjected to further breeding tests as well as a
histological examination. The theoretical hypotheses concern-
ing species crosses have been thoroughly reviewed by other
writers (Babcock and Clausen 1918), but it may not be out of
place to briefly state the particular theories which probably
account for the results considered in this paper.

It has already been pointed out that *H. vulgare* and *H. mu-
ranium* differ in a large number of morphological characters.

H. muranium may be considered as a monotypic species, and the slight variations which are found in the species are undoubtedly due to the effects of the environment and would be classed as non-heritable variations. *H. vulgare*, on the other hand, is a polytypic species consisting of many varieties which differ in a number of morphological characters. Most of the factors which condition the characters of *vulgare* display har-
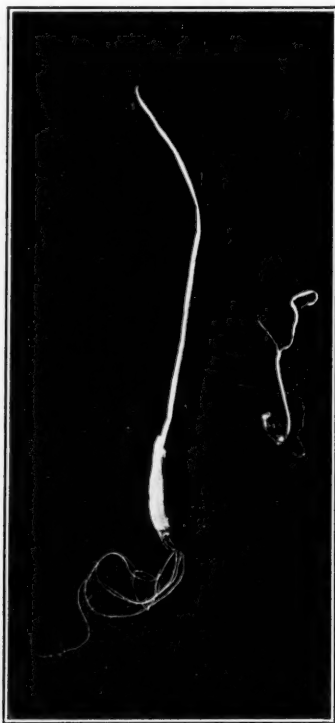

FIG. 2. F₁ species hybrids between *Hordeum vulgare* and *Hordeum muranium* at the stage of growth at which development ceased.

monious interrelations with one another and mendelize in a normal fashion. Several factors involving chlorophyll reduction have been discovered, however, and these genes have been found to be incompatible with the normal functioning of the chromatin system. In these cases after the food material in the seed has been exhausted the seedlings usually die, for the change

in the factors has been too far reaching to give a normal functioning reaction system.

This brings forth the theory of reaction systems which has been thoroughly reviewed by Goodspeed and Clausen (1917a). The purpose of the discussion in the preceding paragraph was to show that both *vulgare* and *muranium* possess a normal reaction system, and second that a normal reaction system may sometimes be disturbed by lethal factors. When we attempt to combine two distinct reaction systems, however (and the distinct morphological characters of the two species as well as the breeding test would indicate that the two species possessed different reaction systems) an inharmonious group is often formed which fails to function in a normal fashion. In the case of chlorophyll reduction there is one or at most only a few factors disturbing the reaction system. In the case of species crosses there are a number of factors, which in all probability differ qualitatively, coming from two distinct reaction systems and these often fail to harmonize. The results are often similar, however, for the differences between the reaction systems of *vulgare* and *muranium* are so profound that the resulting system is not able to function after the food material in the seed is exhausted.

The type of species cross described in this paper is quite similar to the species cross between *Crepis capillaris* and *Crepis tectorum* recently described by Babcock and Collins, 1920. The two species of *Crepis*, besides differing in several morphological characters, were found to differ in chromosome number. Reciprocal crosses gave equivalent results, or the dominance of *tectorum* cotyledon characters in F_1 accompanied by hybrid vigor. The F_1 seedlings died, however, in every case at the end of the cotyledon stage. Cytological examination revealed a complete lack of order in the cell systems, and as a result these systems failed to function and development ceased. The species cross in barley involves slightly greater contrasts perhaps than those in *Crepis* but both give nearly parallel results.

There are, as has been pointed out by others, all degrees of incompatibility of reaction systems in species crosses. The range of compatibility includes cases of complete or nearly complete fertility, as in the species crosses in *Antirrhinum* · (Baur and Lotsy), examples like those found in *Nicotiana* (Goodspeed and Clausen 1917b) where the fact of incompatibility does not become evident until the fertilization of the F_1 plants, and

finally we have species which exhibit complete incompatibility by refusing to cross with one another. The range includes many intermediate conditions like those found in *Crepis*, which nearly approach complete incompatibility. The cross between *H. vulgare* and *H. muranium*, then, is well down the scale and can be grouped in the class with the two species of *Crepis* as showing nearly complete incompatibility.

<div align="right">Walter Scott Malloch</div>

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NOTATIONS

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A NOTE ON UNILATERAL REACTIONS OF THE MELAN-'OPHORES OF THE HEAD IN FISHES

In most discussions of the physiology of the chromatophores of fishes it is apparently assumed that the reactions are strictly bilateral, *i.e.*, synchronous on the two sides. The writer, however, has lately observed a number of cases in which the reaction was either unilateral or imperfectly bilateral.

Upon death, the melanophores of one side of the head in some cases become all ''contracted'' to the extreme, while those of the other side become widely ''expanded.'' As a result, one side of the head becomes very pale, the other side blackish, the two areas being abruptly opposed along the mediodorsal line. This notable

color change at death has been observed by the writer in an adult pike (*Esox lucius*); in a young-of-the-year of the shiner (*Notropis cornutus*); and in embryonic and larval whitefish (*Coregonus clupeaformis*) and lake-herring (*Leucichthys ontariensis*).
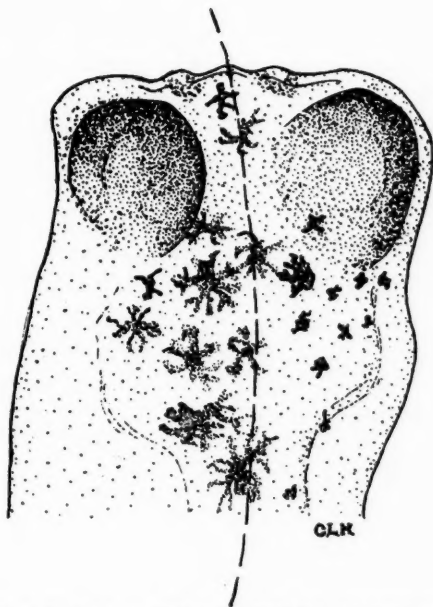
Dorsal Aspect of Head of an Embryonic Whitefish (*Coregonus*), to Illustrate the Unilateral Reaction of the Melanophores.

This phenomenon is not confined to death, however, as the following observations demonstrate. A nuptial male of *Pimephales notatus* (a minnow in which the head becomes densely charged with black pigment during the breeding and nesting activities), apparently normal in respect to its eyes and other structures and functions, found guarding its eggs, had one side of the head abruptly pale. Similarly embryonic and larval coregonine fishes were repeatedly observed to have the melanophores expanded only on one side of the head during life. In the case of the male *Pimephales*, no change in the pigmentation of the head was noted while the fish was being observed for several minutes, nor upon its capture, death or preservation.

In other cases the unilateral reaction of the melanophores was less permanent, appearing as a transient phenomenon; due perhaps, to a differential reaction rate of the chromatophores of the two sides. Two experiments[1] illustrative of this point may be cited.

1. A live, normal, apparently healthy embryo of the lake whitefish (*Coregonus clupeaformis*), developed approximately to the hatching stage, was found to have the dorsal melanophores considerably "expanded" on the head, slightly expanded on the body. Following the removal of the egg envelope, under approximately unchanged conditions, these color-cells "contracted" in this order: (1) body, (2) left side of head, (3) right side of head. Still under similar conditions, the cephalic melanophores again expanded, those of the left side most widely. No further change could then be induced, even by rather intense light-heat stimulation, until the left eye was dissected out and the embryo again held before the light. Reaction occurred at once only on the left (now the blinded) side, the lateral melanophores contracting more rapidly and more completely than the inner ones; as in the first instance, reaction followed (some time after the removal of the stimulus) on the right side, the melanophores contracting in the same order as on the left side.

2. A similar embryo of the same species had the dorsal melanophores of the head well expanded when removed from its egg envelope. The pigment granules of all melanophores on the right side then rapidly migrated into the center of the cells, under observation. No reaction occurred on the left side, even following stimulation with a bright light, although this caused first a partial contraction and then a re-expansion of the right chromatophores. Reaction on both sides was finally accomplished by sudden transfer of the eggs from water near room-temperature (about 25°) to water at 1.7° C., but even in this case the contraction was more complete and rapid on the right than on the left side.

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